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Review

Artemisia annua as a self-reliant treatment for malaria in developing countries

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ABSTRACT

Malaria is a vector-borne infectious disease caused by the protozoan *Plasmodium* parasites. Each year, it causes disease in approximately 515 million people and kills between one and three million people, the majority of whom are young children in sub-Saharan Africa. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Due to climate change and the gradual warming of the temperate regions the future distribution of the malaria disease might include regions which are today seen as safe. Currently, malaria control requires an integrated approach comprising of mainly prevention, including vector control and the use of effective prophylactic medicines, and treatment of infected patients with antimalarials. The antimalarial chloroquine, which was in the past a mainstay of malaria control, is now ineffective in most malaria areas and resistance to other antimalarials is also increasing rapidly. The discovery and development of artemisinins from *Artemisia annua* have provided a new class of highly effective antimalarials. ACTs are now generally considered as the best current treatment for uncomplicated *Plasmodium falciparum* malaria. This review gives a short history of the malaria disease, the people forming a high risk group and the botanical aspects of *A. annua*. Furthermore the review provides an insight in the use of ART and its derivatives for the treatment of malaria. Its mechanism of action and kinetics will be described as well as the possibilities for a self-reliant treatment will be revealed. This self-reliant treatment includes the local production practices of *A. annua* followed by the possibilities for using traditional prepared teas from *A. annua* as an effective treatment for malaria. Finally, HMM will be described and the advantages and disadvantages discussed.

Contents

Abbreviations: ART, artemisinin; ACTs, artemisinin-based combination therapies; CNS, central nervous system; DHA, dihydroartemisinin; HMM, home-based management for malaria; HIV, human immunodeficiency virus; IC_{50}, median inhibitory concentration; MMV, medicines for malaria venture; SERCA, plasmodial sarcoplasmic/endoplasmic calcium ATPase; RDTs, rapid diagnosis tests; TCM, traditional Chinese medicine; WHO, World Health Organization; WHO/TDR, World Health Organization/Special Programme for Research and Training in Tropical Diseases; DFID, UK Department for International Development; USAID, United States Agency for International Development.

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1. Introduction

Malaria is an age-old disease which has had a large influence on the economies and development of nations for millennia. It was also responsible for crushing defeats during wars as sometimes more soldiers were killed by malaria than during battle. It was first thought to be caused by poisonous vapors from standing water or swamps and therefore the Italians called the disease "mala–aria" which means "bad air" ("mala" = bad and "aria" = air). The two terms became together known as "malaria" which is still used today to describe the disease. Historically malaria occurred in many parts of the world but mortalities steadily decreased from 1900 to 1997 all over the world except in sub-Saharan Africa where there was a significant increase in mortalities (Table 1) (Snow et al., 2001; Carter and Mendis, 2002; Barnes and White, 2005).

Today we know that malaria is a vector-borne infectious disease caused by the protozoan Plasmodia parasites. There are four types of *Plasmodium* species namely *P. falciparum*, *vivax*, *malariae* and *ovale* while the vector carrying and transmitting the disease is of the female *Anopheles* mosquito species (Toure et al., 2004). It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, it causes disease in approximately 515 million people and kills between one and three million people, the majority of whom are young children in sub-Saharan Africa (Snow et al., 2005). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development (Mboera et al., 2007). Mortality, currently estimated at over a million people per year, has risen in recent years, mainly because of increasing resistance to antimalarial medicines and to a lesser extent due the increase of HIV-AIDS (Snow et al., 2001).

Table 1
Mortalities from malaria in the different regions of the World in different years (Carter and Mendis, 2002).

Region	Year	Total no. of deaths from malaria	% of all deaths which are due to malaria
Europe and North America	1900	80,000	0.8000
	1997	20	0.0001
Caribbean, Central and South America	1900	42,000	2.0000
	1997	4,000	0.0500
Asia, China, and Western Pacific	1900	2,800,000	9.0000
	1997	65,000	0.1000
Sub-Saharan Africa	1900	210,000	6.0000
	1997	990,000	9.0000
World excluding sub-Saharan Africa	1900	2,900,000	8.0000
	1997	69,000	0.0800

Malaria control requires an integrated approach comprising of mainly prevention, including vector control and the use of effective prophylactic antimalarials, and treatment of infected patients with effective antimalarials (Olumnese, 2006). The antimalarial chloroquine that was in the past a mainstay of malaria control is now ineffective in most *P. falciparum* malaria areas and resistance to other antimalarials is increasing rapidly (Talisuna et al., 2007).

The discovery and development of ART from *Artemisia annua* L. (Asteraceae) have provided a new class of highly effective antimalarials, and have already influenced the therapy of malaria (Sriram et al., 2004; Haynes, 2006; Hsu, 2006; Bosman and Mendis, 2007). ACTs are now generally considered as the best current treatment for uncomplicated *P. falciparum* malaria (Olumnese, 2006). ACTs are also not likely to be affected by resistance in the near future if everyone has access to this therapy (Yeung et al., 2004).

Most of the malaria infections especially in sub-Saharan Africa are treated in peripheral health centers or remote villages, where facilities are limited. The aim of any effective malaria management strategy is therefore to provide a simple and inexpensive treatment that can be applied effectively in most settings.

This review briefly discusses the different types of malaria and the people who are the most vulnerable to become infected with the disease. In addition we will briefly discuss the Artemisia taxonomy after which we will mainly focus on the use of ART and its derivatives for the treatment of malaria. The mechanism of action of ART and its kinetics will be described and the possibilities for a self-reliant treatment for malaria will be revealed. The local production practices of *A. annua* will be discussed, followed by describing the possibilities for using traditional prepared teas from *A. annua* as an effective treatment for malaria. Finally, the HMM initiative will be described and the advantages and disadvantages discussed.

2. The disease

2.1. Malaria

Malaria is the most important parasitic disease of mankind. It has a serious impact on the health and on the economic welfare in the tropical world, including parts of America, Asia and Africa. Most malaria related deaths occur in sub-Saharan Africa, where there is hardly any access to antimalarial drugs (Breman et al., 2004). The occurrence of malaria depends mainly on climate factors such as temperature, humidity, and rainfall. Temperature is particularly critical as temperatures below 20 °C will cause the malaria parasites not to complete their life cycle, and can therefore not be further transmitted (CDC, 2004). Malaria is caused by infection of red blood cells with parasites of the genus Plasmodium. The parasites are

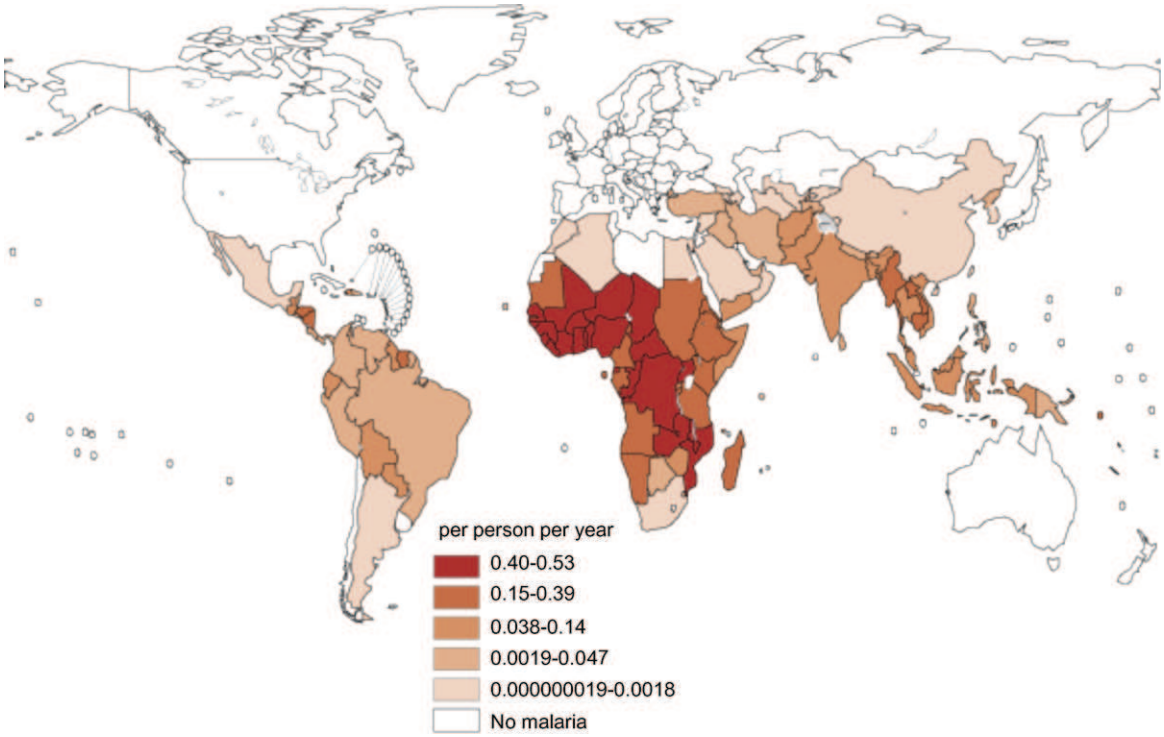


Fig. 1. Global malaria incidence rate in 2006 (WHO).

transmitted into the human host by a feeding female Anopheline mosquito.

Four types of the plasmodium parasites can infect humans of which *P. falciparum* and *P. vivax* are the most important. *P. vivax* has also the potential to cause relapse after a number of years due to persistent liver stages.

- *P. falciparum* is the most dangerous and lethal form of the plasmodium parasites as it has the highest rates of complications and mortality. It accounts for 80% of all malaria infections and 90% of malaria related deaths. Infection with this parasite can lead to death within hours to days (Trampuz et al., 2003).
- *P. malariae* causes benign malaria. Benign malaria can be described as malaria that should not become life threatening as opposed to the term malignant, which means life threatening or a worsening condition. This type of parasites has the longest incubation period, namely 14 days, but can result in chronic infections that last for years (Siala et al., 2005; Mueller et al., 2007).
- *P. ovale* is related to *P. falciparum* and *P. vivax*, but relatively rare and less dangerous. *P. ovale* also has persistent liver stages which can result in relapse after several months (Chin and Coatney, 1971).
- *P. vivax* is the cause of benign, but recurring malaria. Infections are seldom fatal. It is more tolerant of lower temperatures and is therefore more present in cooler regions. Due to persistent liver stages, this parasite can cause relapse up to 5 years after infection (Pukrittayakamee et al., 2004).

Malaria can be classified in two types namely uncomplicated and severe malaria, according to the symptoms and consequences. The classical symptom of uncomplicated malaria is cyclical occurrence of sudden coldness, followed by rigor and then fever and sweating lasting 4–6 h, occurring every 2 days in *P. vivax* and *P. ovale* infections, and every 3 days for *P. malariae* (Collins and Jeffery, 2007). *P. falciparum* can have a recurrent fever every 36–48 h or a continuous fever. In acute *P. falciparum* malaria, there is a continuum from mild-to-severe malaria. The objective of treating uncomplicated malaria is to cure the infection to prevent progression to severe malaria (Bell and Molyneux, 2007). Severe malaria, caused by *P. falciparum*, causes a mortality rate of ~100% if left untreated (Trampuz et al., 2003). With antimalarial treatment the mortality is 15–20%. Symptoms are splenomegaly, severe headaches, cerebral ischemia, cerebral malaria, hepatomegaly, hypoglycemia and haemogobinuria with renal failure and subsequent coma and death. The primary objective of the treatment in the case of severe malaria is to prevent patients from dying and therefore the avoidance of recrudescence and of minor side effects are secondary. With recrudescence the number of parasites in the blood drops below detectable limits and can remain in the blood for a number of months without causing any symptoms. The term relapse refers to the reactivation of the hypnozoite stage in the liver.

2.2. Special high risk groups

People living in malaria regions which are regularly exposed to malaria build up a partial immunity against malaria. This immunity however disappears when they leave that specific area. Travelers or tourists visiting these areas are therefore at a high risk of contracting malaria as they have no partial immunity against the parasite. Research has furthermore resulted in the identification of two important groups who are particularly vulnerable to malaria: pregnant women and very young children (Abdullah et al., 2007; Brooker et al., 2007; Menendez et al., 2007). Malaria can have serious consequences for mother and baby. In high transmission areas, pregnant women are at high risk of developing severe malaria, spontaneous abortion, stillbirth or premature delivery. Infected pregnant women are often asymptomatic but parasitaemia can cause maternal anaemia and low birth weight, a leading cause of infant morbidity and mortality (Dellicour et al., 2007). Malaria causes over 850,000 child deaths every year, mostly in Africa and Asia (Fig. 1). In Africa one in five child deaths are due to malaria (Crawley and Nahlen, 2004). Very small amounts of antimalarials

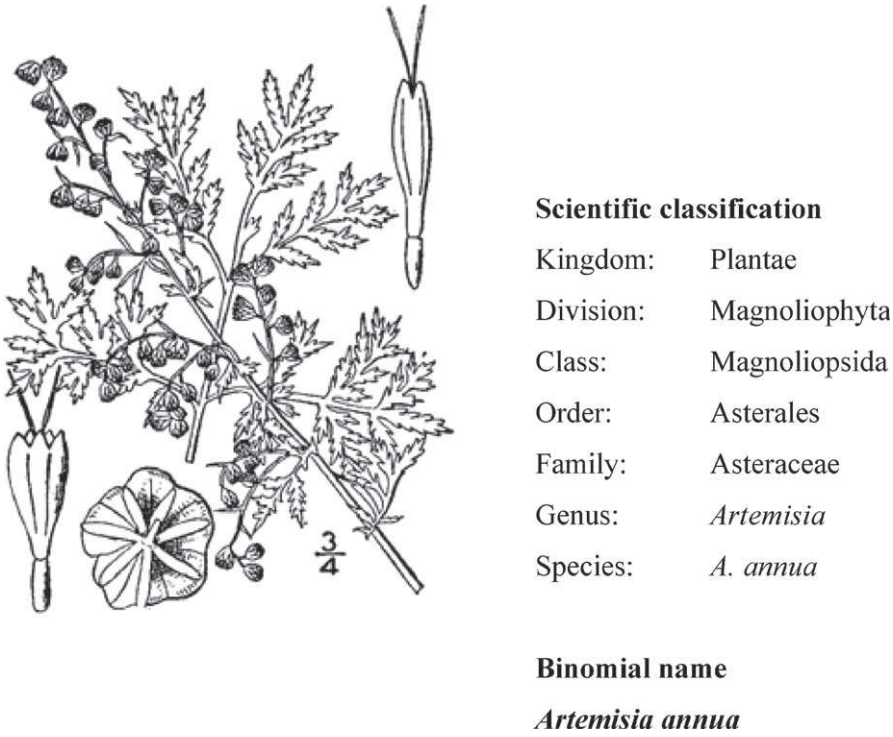


Fig. 2. Drawing of *A. annua* with its scientific classification.

have been discovered in breast milk (AlKadi, 2007). The amount of transferred drug is not harmful to the infant, but also not sufficient enough to provide protection against malaria. Infants who need treatment should therefore obtain the recommended dosages (AlKadi, 2007). Furthermore, elderly and HIV patients are of greater risk to develop adverse effects. Increasing numbers of people in malaria areas are living with HIV infection. If HIV progresses and immunosuppression worsens, the manifestations of malaria also worsen. The symptoms, severity and consequences of malaria in HIV patients are worse than in patients who only have malaria. The treatment failure rates are also higher, due to reduced immunity. There is however insufficient evidence to recommend modifications to antimalarial treatment regimens in patients infected with HIV (Olumnese, 2006).

3. *Artemisia annua*

3.1. Botanical aspects

Artemisia annua belongs to the class of flowering plants Magnoliopsida (Fig. 2). The family of Asteraceae is the second largest family of flowering plants in the world. *Artemisia* is a large, diverse genus of plants with between 200 and 400 species, and comprises hardy herbs and shrubs. *A. annua* is an annual shrub of 50–150 cm in height. The shrub grows in temperate climates and is most widespread in China and Vietnam, but is also cultivated in East Africa, the United States, Russia, India, Brazil and some other countries (Bhakuni et al., 1988, 1990). The reproduction of the shrub occurs by insects, self-pollination, and wind distribution (Bown, 1995).

3.2. Artemisinin content

Except for *A. annua*, ART is also found in *Artemisia apiacea* Hance. and *Artemisia lancea* Vaniot., but only in minor quantities (Hsu, 2006). The main artemisinin-related compounds found in *A. annua* are ART, arteanniun B, and artemisinic acid. Up to 42% of the total ART content is found in the upper leaves, where it accumulates in the glandular trichomes of the leaves (Bhakuni et al., 1988, 1990). The total amount of ART found in different varieties of *A. annua* is between 0.01 and 1.4 wt% based on dry leaf mass (Delabays et al., 2001). This variability can partly be explained by different extraction methods, different collection periods, different sample preparation, and different environmental influences. Another important aspect that may influence the variation is the different analytical equipment and methods employed in order to quantify ART. Unpublished results from our laboratories have confirmed this. The ART concentration of leaves from one plant may also vary in concentration, with the upper leaves containing more ART than the middle or lower leaves (Delabays et al., 2001). The ART content in the plant varies during the season with the highest quantities usually present just before flowering (Liu et al., 2006). However, plants grown under Swiss conditions, resulted in the highest ART concentrations found at the end of August, independent of the developmental stage (Delabays et al., 2001). Genetic variation also influences the ART content.

Hybridized characterized genotypes are hard to produce, because of the numerous flowers of *A. Annua* which results in self-pollination. At present, a breeding program has been completed in Switzerland and has resulted in a cultivar with an exceptionally high yield of ART, called “Artemis” (Golenser et al., 2006). As the plant is annual, the clones of this cultivar are stored *in vitro*.

3.3. History of A. annua as traditional remedy

The Chinese name for *A. annua* is *qing hao*, which has a similar meaning as the words ‘green herb’. In the history of *qing hao*, *A. annua* and *A. apiacea* (also containing small amounts of ART), were not recognized as different species. The separation into two different species of *qing hao* and *huang hua hao* became perma-

nent in 1518. Noteworthy, strictly speaking, *A. annua* is *huang hua hao* and not, as is commonly assumed, *qing hao* (Hsu, 2006). The first description of the Chinese herb *A. annua* dates back to the year 168 B.C. (Efferth, 2007). In the fourth century, Ge Hong described a method of preparing *qing hao*, although it was not until 1596, due to Li Shizhen, that his method was recorded within the encyclopedic *Classified Materia Medica*. Ge Hong's method consisted of soaking the fresh plant in water, and then wringing out the whole plant and ingesting its juice in its entirety. The soaking of the entire plant in water and its subsequent wringing out might have resulted in an emulsion of water, flavonoids and other etherical oils contained in the stem and leaves, which may have facilitated the extraction of the artimisinin compounds (Hsu, 2006). *Classified Materia Medica* was a forerunner of the Chinese *Material Medica.* According to this handbook, tea-brewed leaves were used to treat (intermittent) fever, chills, lice, wounds and 'lingering heat in joints and bones' (bone fractures, exhaustion due to fevers). Furthermore, *A. annua* was recommended as a food-supplement and was considered to have longevity-enhancing properties, which may be an indirect effect of the consequence of its antimalarial effects. Later, it was also used to treat acute convulsions, which in China were related to pollution through contact with the dead and possession by demons. This may be related to cerebral malaria. During the Vietnam War (1965–1975) the Chinese government started an antimalarial research program to search for traditional Chinese medicinal plants to support the Vietnamese army. As a result, ART (*qinghaosu*) was identified in 1972 as the active antimalarial constituent of *A. annua*. Today, ART and its derivatives are widely used as antimalarials against drug resistant *Plasmodium* strains, cerebral malaria, and malaria in children. Besides treatment of malaria, ART is also used to treat infections of *Schistosoma* spp., *Pneumocystis carinii*, *Toxoplasma gondii*, human cytomegaloviruses, *Herpes simplex* viruses, and hepatitis B and C. Artemisinin has also been shown to have *in vitro* activities against certain tumor cells (Efferth, 2007).

3.4. ART derivatives

Pure ART has a low solubility in water and oil, and thus can therefore be administered orally (Barradell and Fitton, 1995), rectally and intramuscularly. Unfortunately, oral administration is often not possible in patients with severe malaria, due to extreme vomiting. To resolve this problem, several semi-synthetic ART derivatives (Table 2) have been developed (Golenser et al., 2006). These derivatives include the water-soluble artesunate and the oil-soluble artemether and arteether. Artemisinin-related compounds that are found in *A. annua* are arteanniun B and artemisinic acid. Artemisinic acid is the precursor for ART in the biotransformation pathway, and is an important precursor in the development of a semi-synthetic route to ART (Mueller et al., 2000; Kim and Sasaki, 2004; Lapkin et al., 2006). The peroxide bridge is the active group for antimalarial activity and therefore it does not need the complex ring structure (Meshnick et al., 1996). This makes it easier to synthesize simplified analogs as trioxanes (Table 2). Fenozan 50F is a promising compound with activity against a variety of resistant strains (Peters et al., 1993a,b; Fleck et al., 1997). It is effective against the gametocytes sporozoites stages of *P. falciparum* in vivo (Peters et al., 1993c). The synthetic analogue, WR 279137 is also effective against *P. falciparum* in monkeys (Posner et al., 1994).

3.5. Mechanism of action

All ART compounds induce a very rapid reduction of parasitaemia, starting almost immediately after administration (Balint, 2001). ART and its derivatives kill all stages of parasites by interacting with haem to produce carbon-centered free radicals that alkylate proteins and damage the micro-organelles and membranes of the parasite. But despite the reliance on ART drugs, their exact mechanism is still unresolved. However, the complex ring structure is not necessary for antimalarial activity, only the peroxide bridge (C–O–O–C) is (Meshnick et al., 1996; Golenser et al., 2006). The complex ring structure might not be important for the action of ART but it might be essential for the stability of the molecule in order for it to reach its site of action. Comparison of drug activity against eukaryotic cell lines, relative to the activity against malaria parasites was performed *in vitro* to predict drug specificity against *P. falciparum*. The effects of ART and ART analogues were examined on chloroquine-sensitive and chloroquine-resistant strains. The IC_{50} of parasite strains differed when treated with chloroquine (16.5 nM against sensitive strains vs 232.6 nM against resistant strains). However, there were no differences between the IC_{50} values of ART (17.0 nM vs 14.5 nM) when comparing the chloroquine-sensitive and non-sensitive strains. These results suggest a specific mechanism of action of artemisinins against *P. falciparum* (Avery et al., 2003). Two mechanisms of action are proposed.

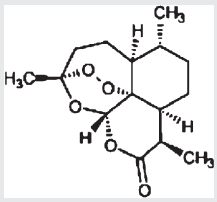
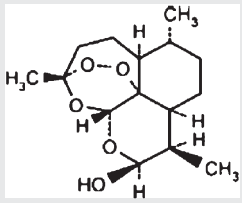
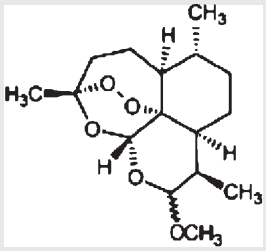
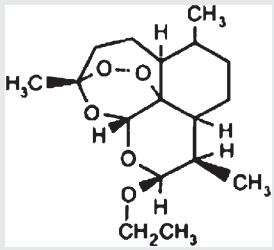
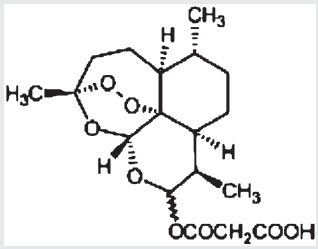
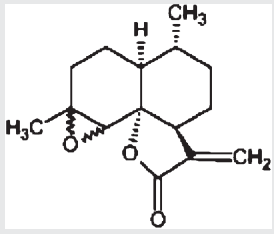
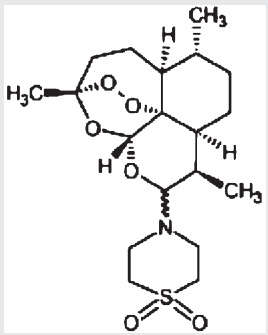
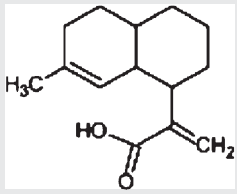
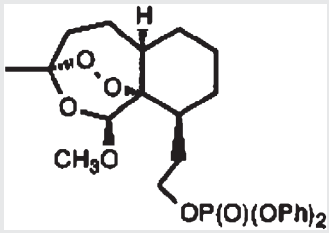
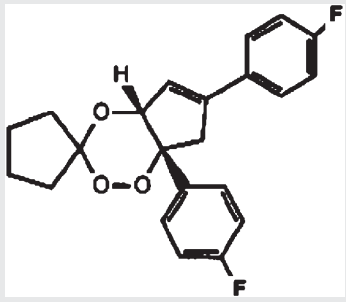
The first proposed mechanism is interference with the SERCA (Eckstein-Ludwig et al., 2003; Liu et al., 2006). ART is structurally similar to thapsigargin, an inhibitor of SERCA. Therefore, it was hypothesized that artemisinins specifically inhibit SERCA. SERCA is responsible for the maintenance of calcium ion concentrations, which is important for the generation of calcium-mediated signaling and the correct folding and post-translational processing of proteins. PfATP6 is the only SERCA-type Ca^{2+} ATPase in *P. falciparum.* ART completely inhibits specific PfATP6 activity. Addition of thapsigargin, a structural similar compound, resulted in antagonism (Eckstein-Ludwig et al., 2003). Modeling of PfATP6 demonstrated that artemisinins bind to the protein by hydrophobic interactions while leaving the peroxide bonds exposed. This allows cleavage of the peroxide bridge by iron to generate carbon-centered radicals, leading to enzyme inactivation and parasite death. This theory is supported by mutations in SERCA resulting in differences in sensitivity to artemisinins. This, therefore, may be a mechanism that can result in resistance. Noteworthy is that this proposed mechanism results in stereoselectivity. However, enantiomers of trioxanes show equivalent levels of activity against *P. falciparum*.

The second proposed mechanism of action is the production of reactive species (Fig. 3) (van Agtmael et al., 1999; Ittarat et al., 2003). The action of ART derivatives is different from that of the other antimalarial drugs, although both artemisinins and quinolines interact with haem. Artemisinins have a very fast action and parasites clearance times are much shorter than with other antimalarials. Artemisinins act on late trophozoite, schizont, early trophozoite and ring stages. ART is only active on blood-stage parasites and does not affect liver-stage parasites or stages within the mosquito. It does act on gametocyte development, resulting in decreased transmission. During the blood-stage phase of the parasite, more than 70% of the haemoglobin within the infected erythrocyte is digested. Haem is released, which is toxic for the parasite and therefore neutralized by polymerization into haemazoin. It was found that haem or Fe^{2+} catalyses the opening of the peroxide bridge in ART, leading to the formation of free radicals. The initially formed oxygen radicals rearrange to primary and secondary carbon-centered radicals intermediates in the formation of known metabolites. These intermediates are involved in the alkylation of proteins. Both the proposed mechanisms of action depends on the activation of the peroxide group leading to the production of reactive species.

3.6. Metabolism of ART

Metabolism of ART and its derivatives is mediated by cytochrome P450 enzymes CYP2C19 and CYP2B6, which are asso-

Table 2
Artemisinin and its (semi-) synthetic derivates.

Artemisinin		Dihydro-artemisinin (DHA)	
Artemether		Arteether	
Artesunate		Arteanniun B	
Artemisone		Artemisinic acid	
Trioxanes			
Fenozan 50F		WR 279137	

ciated with first pass drug metabolism. While ART is converted to DHA, its derivatives artesunate, artemether and arteether are also metabolized into DHA, which has an effect at least as potent as its parent compounds (Balint, 2001; Burk et al., 2005). ART drugs have a short half life (although there is a significant difference in the half lives of the different derivatives with artesunate being metabolized almost immediately, while artemether and arteether are being metabolized more slowly), while DHA has a half life of ~1 h. Treatment with artemisinins causes reduction of the parasite below detectable limits, without eliminating all parasites; the few parasites not killed by the drug continue to grow leading to a recurrence (recrudescence) of malaria symptoms. In order to completely eliminate the parasites and prevent the emergence of resistant *P. falciparum*, combinations with other, longer-acting drugs are necessary. In healthy, normal volunteers, orally administered ART is metabolized in the liver. Due to the first pass effect the bioavailability is only 32% (Titulaer et al., 1990; Meshnick et al., 1996).

3.7. Toxicity of ART

Drug toxicity is viewed differently depending upon whether the clinical indication is complicated or uncomplicated malaria. For the

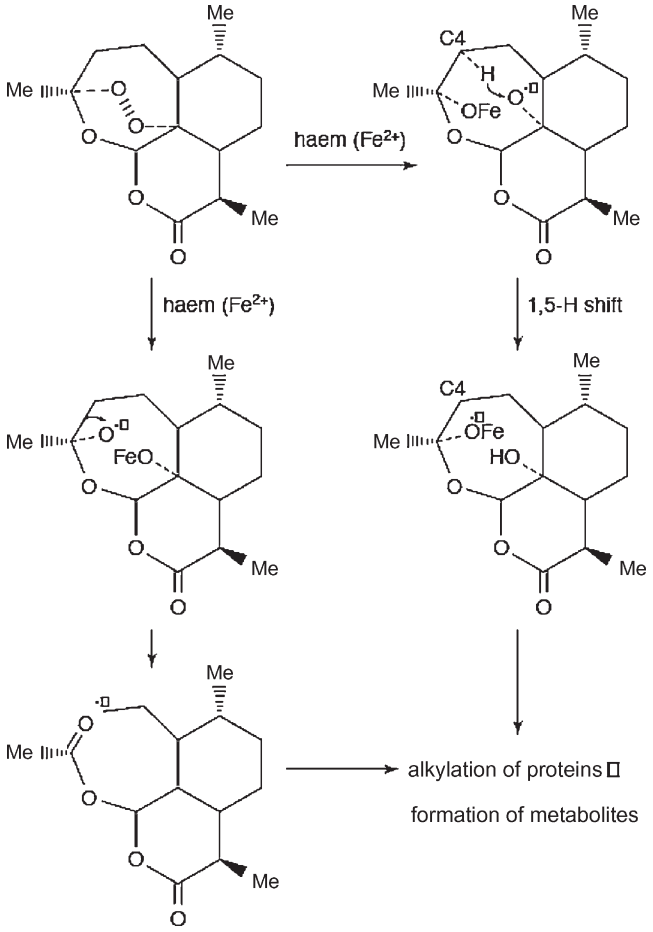


Fig. 3. One of the proposed mechanisms of action. Upon reduction of the peroxide bridge by Fe^{2+}, two radical anions can be formed. In one of these a 1.5-H shift between the oxygen radical and a hydrogen atom at C_4 occurs with formation of a carbon radical and subsequent formation of a presumed epoxide intermediate which is electrophillic and can react with proteins. Rearrangement of the other oxygen radical intermediate gives rise to a primary radical involved in the alkylation of haem and parasite proteins leading to parasite death (van Agtmael et al., 1999).

treatment of *P. falciparum*, which has a high mortality if untreated, a greater risk of adverse reactions is inevitable. Compared to other antimalarials, ART and its derivatives have a good safety profile and is remarkably non-toxic. Adverse effects have been reported in artemether–lumefantrine treatment, were abdominal pain, anorexia, nausea, vomiting, diarrhea and CNS involvement (headache and dizziness) occured. Oral artemisinins, alone, are well tolerated (AlKadi, 2007).

4. Self-reliant treatment with *A. annua*

In Chinese medicine *A. annua* was traditionally prepared as a tea to treat fever and chills (Hsu, 2006). These symptoms can be seen as malaria related symptoms and this was proven by the fact that hundreds of years later the active component, ART, was isolated. This poses the important question "Can this holistic traditional method be used worldwide to effectively treat malaria without the emergence of drug resistance?"

4.1. Production of ART

Since the discovery of the antimalarial properties of *A. annua*, the large-scale production of this species has been on the increase. Usually, once the active chemical structure has been elucidated, scientists investigate the chemical synthesis of the compound. The first total synthesis of the molecule was reported by Schmid and Hofheinz (1983). Since then, several teams reported different pathways for the synthesis of ART, but all of them had many steps and a low overall yield. None of these complex synthetic pathways provides a feasible method for the large-scale production of ART. At the moment, its extraction from *A. annua* plants remain the only source for the drug (Avery et al., 2003). The production of ART in cell, tissue and intact plant cultures of *A. annua* has been widely investigated (Nair et al., 1986; Souret et al., 2003; Liu et al., 2006; Ro et al., 2006; Baldi and Dixit, 2008). Shoots only produce ART when the roots are still present with a maximum of 0.28% dry mass (Putalun et al., 2007). Most progress in the production of ART is expected from the selection and breeding of high ART yielding cultivars, although controlled cultivation is hard because of the numerous flowers, *A. annua* usually results in self-pollination. It has also been proposed to synthesize ART from its precursors, namely artemisinic acid and arteanniun B (Ro et al., 2006). The overall demand of artemisinins has increased from 22,000 treatment courses in 2001 to an estimated 200 million in 2008 (WHO, 2008 update). Due to the shortage of ART, the possibility to grow *A. annua* locally (especially in Africa) might compensate for the lack in supply of ART by providing its own raw materials from which ART can be produced (Mueller et al., 2000).

4.2. Cultivation

Mueller et al. (2000) have described the cultivation of the *A. annua*, *"Artemis"* cultivar in the tropics, in three locations in the Democratic Republic of Congo. The cultivation method employed resulted in large plants with an average height of 2–2.5 m, 7–8 months after germination which yielded 100–200 g of dried leaves per plant. In comparison, 250–300 g of dried leaves per plant are reported after professional cultivation in Europe (Mueller et al., 2000). Leaves produced at altitudes of 1650 and 2000 m showed 0.63% and 0.70% ART per dry mass. In comparison, the leaf material of plants cultivated professionally showed a content of 0.58%. The WHO estimated that they will require 200 million adult courses of ART. One treatment course of Coartem (Artemether + Lumefantrine) consists of between 16 and 24 tablets, each containing 20 mg of artemether.

On the basis of this information it is possible to estimate the required land area of *A. annua* plantations to satisfy the annual demand. The chemical yield of artemether from ART is 60%, the global requirement is ca. 96,000 kg. The average ART content, locally cultivated, is 0.7% but the amount of harvested leaves varies greatly. A study based on field trials in Vietnam reported a yield up to 7000 kg h^{-1} leaves (dry mass), yielding ca. 30 kg h^{-1} ART at a plant density of 20 plants m^{-2}. Another trial harvested young leaves from the same plants up to four times, with a maximum yield of 77.5 kg h^{-1} of ART. Generally, it is believed that large farms using best practices are achieving 4000–5000 kg h^{-1} of leaves (dry mass) while small holders manages only about 1000 kg h^{-1}. Based on these values, the annual worldwide area of *A. annua* plantations should be between 4300 and 20,000 ha (from Lapkin et al., 2006). Such an area of land is fairly small but given the uncertainty over the future demand due to potential development of vaccines, decrease in price of synthetic alternatives, and development of resistance to artemisinins it will remain an economically risky undertaking. The cultivar "Artemis" was used in these experiments as this cultivar contains a high and relatively reproducible amount of ART in the leaf (Delabays et al., 2001). This is necessary to determine the dose according to the leave weight. Based on these facts, it can be stated that the local production of ART from *A. annua* is currently possible. This is the first step in a self-reliant treatment of malaria.

Table 3
Results of trial of Rath et al. (2004). Method C is considered the best method, for its high efficiency of extraction.

Preparation method	Amount of *A. annua* dried leaves (g)	Artemisinin concentration in tea preparation (mg/L)	Efficiency of extraction (%)
A	5.0	57.5	83
	9.0	88.2	71
B	5.0	36.5	53
	9.0	37.8	30
C	5.0	60.0	86
	9.0	94.5	76

4.3. Tea from A. annua as antimalarial

In TCM, *A. annua* was used against fevers and chills that we today associate with malaria. It was also recommended as a food supplement, because it was considered to have longevity-enhancing properties. In TCM, *A. annua* was used to prepare a tea (Blanke et al., 2008) and if it contains effective amounts of ART, it might be used today as a self-reliant treatment of malaria. Local production of *A. annua* can result in an increased access to the antimalarial in rural areas and this will result in a decrease in mortality of *Plasmodium* infections. In this section an overview of the preparation, efficacy and safety of traditionally prepared *A. annua* tea is presented.

4.4. Preparation of traditional tea

During a trial performed by Rath et al. (2004), the upper leaves of a *A. annua* hybrid (*A. annua L.* cv. *Artemis*) were used to prepare a tea. The plants were cultivated under controlled conditions in Germany. The complete plant was harvested and air dried after which the dried leaves were used to prepare teas using two different quantities (5 and 9 g of leaves) for each of the following preparation methods.

A. After addition of boiling water to the leaves, the mixture was left to cool to room temperature after which the leaves were removed by filtration.
B. The leaves were boiled for 30 min in water after which the mixture was left to cool to room temperature, and the leaves subsequently removed by filtration.
C. After addition of boiling water to the leaves, the mixture was briefly stirred and the container was covered for 10 min. Subsequently, the leaves were removed by filtration and squeezed gently to release residual water. The tea was allowed to cool to room temperature.

The ART content in the tea prepared with the three different methods were determined. Results from the trial are shown in Table 3. Method C yielded the highest amount of ART (94.5 mg of ART in 1 l of tea) which corresponds to a 76% extraction efficiency when using 9 g of leaves. When 5 g of leaves are used the tea yielded 60.0 mg ART (86% extraction efficiency). Boiling reduced the yield considerably, presumably due to the known chemical lability of ART.

In a trial performed by Mueller et al. (2000), two methods of tea preparation were compared. The same hybrid, *A. anuua* cv. *Artemis*, was also used during these experiments. The plants were cultivated under the local conditions in the Democratic Republic of Congo. The leaves were harvested and dried and the tea prepared as follows:

A. Boiling water (1 l) was added to 5 g of dried leaves. After brief stirring, the mixture was left to cool for 15 min, and filtered.

Table 4
Results from Mueller et al. (2000).

Amount of material per L water	Method of tea preparation	Artemisinin concentration in tea (mg/L)	Extraction efficiency (%)
Pure artemisinin (250 mg)	A	10.6	–
Leaves (5 g)	A	12.0	41.4
Leaves (10 g)	A	24.5	42.2
Leaves (20 g)	A	32.8	28.3
Leaves (40 g)	A	64.4	27.8
Leaves (5 g)	B	7.2	24.8

Method A: 1 l of boiling water is added to 5–9 g dried leaves of artemisinin, stirred, cooled for 15 min and filtrated. Method B: 5 g of leaves was added to 1 l of water, heated to boiling point, kept boiling for 5 min and filtered. Method A is considered to be the best method.

B. Dried leaves (5 g) were added to 1 l of water and heated to boiling point. The mixture was kept boiling for 5 min after which the leaves were removed by filtration.

Chemically pure ART has a low aqueous solubility, but it was shown during this study that the tea preparations contained higher than expected amounts of ART. This might be caused by the presence of other plant constituents with amphiphilic properties (e.g. flavonoids or saponins) which might improve the solubility of ART in water (Olumnese, 2006). The most efficient method (method A) was adding boiling water to the dried leaves (Table 4).

There are several differences in setup of the two trials which might explain the obtained results. Firstly, the cultivation of the hybrid differed between the trials. Secondly, the leaves were harvested in different ways. In the trial of Räth, the complete plant was harvested and dried while during the trial of Mueller, only the leaves were harvested and dried. Method A of Räth is comparable to the method A of Mueller but during the trial of Räth the tea contained 57.5 mg/l ART, whereas in the Mueller trial the concentration was only 12.0 mg/l. The cause of this difference is not known, but may be due to the differences in harvesting and cultivation practices.

With the use of the *A. annua* tea preparations, the effective plasma concentration threshold of 10 μg/l, above which a growth inhibition of the parasite is observed (Alin and Bjorkman, 1994) was achieved in both trials. Both trials also concluded that extended boiling of the mixture reduced the yield considerably, as this results in the degradation of ART in the mixture. From both trials can be concluded that ART can be extracted from the plant by addition of boiling water.

4.5. Pharmacokinetics and pharmacodynamics of tea from A. annua

The trial of Räth was continued by a non-randomized clinical trial in healthy male Caucasian volunteers. Boiling water was added to nine grams of dried leaves according to method C (yielding 94.5 mg/l of ART). The trial was designed to determine ART plasma concentrations after oral intake of the traditional prepared tea of *A. annua*. The amount of ART in the tea corresponds to only 19% of the prescribed, recommended daily dose of ART in adults (500 mg). The mean plasma concentration–time curve for all 14 subjects after drinking the tea were determined (Fig. 4). ART was absorbed quickly, and maximum concentrations were reached after 30 min (240 ng/ml). In comparison, 500 mg pure ART resulted in a C_{max} of 531 ng/ml after 2.3 h. The C_{max} of the tea preparation was shown to be 40% of the C_{max} of the capsule intake. The bioavailability of ART from the tea preparation was found to be similar to that from ART in capsules. The minimum concentration required

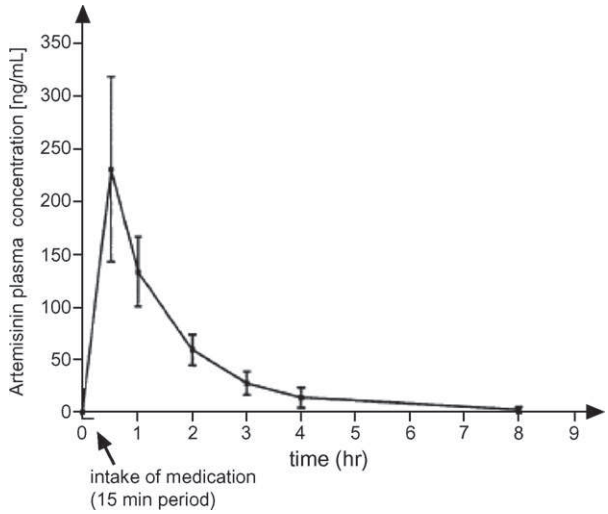


Fig. 4. Artemisinin plasma concentration–time curve after oral intake of 1 l of *Artemisia annua* tea containing 94.5 mg of artemisinin. Data represent the mean ± SD from 14 healthy volunteers.

for growth inhibition of *P. falciparum in vitro* is estimated on 10 μg/l (Alin et al., 1996).

Mueller et al. (2000) continued with a clinical trail in central Africa on malaria patients. Inclusion criteria were: presence of malaria parasites and subjective symptoms. Exclusion criteria were pregnancy, breastfeeding, an age less than 11 years and severe diseases besides malaria (HIV). They also used traditional prepared tea. After 4 days of treatment, blood films were examined and the parasites were counted. Five volunteers were also examined by blood films on each treatment day. The trial was performed at two locations and at each location a different tea preparation was used for the treatment of patients. In the Lwiro region (preparation: no additional boiling of the leaves) the trial resulted in the following dynamics. The parasite count dropped rapidly and parasites were undetectable. Of the 17 persons, 15 reported disappearance of the symptoms while all patients were free of parasites (Fig. 5). The trial performed in the Nebobongo region (preparation: with additional boiling) resulted in 44 patients (92%) with disappearance of the parasite and 37 patients (77%) were free of symptoms. It is however important to note that this study excluded young children who are the most vulnerable to malaria. The older children and adults tested are likely to have build up partial immunity against malaria which means the results cannot be extrapolated to young children which forms a high risk group.

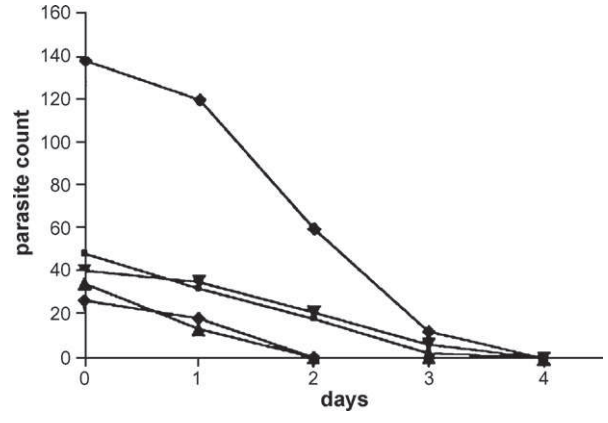


Fig. 5. Parasite counts in thick blood films of five, malaria patients undergoing treatment with *A. annua* tea.

Table 5
Cure rates of artemisinin and quinine (treatment for 7 days) after 7, 14, 28 and 35 days.

Daily dose	Cure rate (%)			
	Day 7	Day 14	Day 28	Day 35
5 g dried leaves	77	57	38	34
9 g dried leaves	70	58	37	30
1500 mg quinine	91	90	86	79

In another trial, also performed by Mueller et al. (2004), the efficacy and safety of *A. annua* tea preparations in the treatment of uncomplicated *P. falciparum* malaria was evaluated. Two doses were used; 5 g dried leaves in 1 l of water a day (corresponding to 47 mg ART) and 9 g dried leaves in 1 l of water a day (94 mg of ART) for 7 days. The control was quinine treatment. The cure rates are shown in the Table 5. The symptoms dropped rapidly but during the follow-up the cure rates in the ART groups decreased while the symptoms reappeared, indicating a high and unacceptable recrudescence rate (reappearance of the disease). In the control group, the recurrence of malaria was less, indicating the observed recurrence was indeed due to recrudescence in the ART group, instead of re-infection. Monotherapy with tea preparations from *A. annua* can therefore not yet be recommended as a treatment option for malaria because of recrudescence, and therefore concerns about possible resistance developing against ART.

5. Combination therapies

5.1. Resistance

Resistance to antimalarials has been documented for *P. falciparum*, *P. vivax* and *P. malariae* although resistance occurring in the latter two species are rare. In *P. falciparum*, resistance has been observed to almost all currently used antimalarials (amodiaquine, chloroquine, mefloquine, quinine and sulfadoxine–pyrimethamine) except for ART and its derivatives. Resistance occurs due to indiscriminate use of antimalarials, which has resulted in mechanisms in the *Plasmodium* strains that make antimalarials ineffective (Golenser et al., 2006). Partly due to this resistance, the mortality of malaria has risen in recent years. Resistance can be prevented by combining antimalarials with different mechanisms of action and ensuring very high cure rates through full compliance and correct dosing regimens (Burk et al., 2005). To counter the threat of resistance of *P. falciparum* to monotherapies, combinations of antimalarials are now recommended by WHO for the treatment of *P. falciparum* malaria. Antimalarial combination therapy is the simultaneous use of two or more drugs, with independent mechanisms of action. Combination therapy should improve efficacy, and delay resistance to the individual components. In the event that a mutant parasite is resistant to one of the drugs, the parasite will be killed by the other drug. Drug combinations that rely on synergy or include a non-antimalarial are not considered as antimalarial combination therapy. Disadvantages of combination therapy are an increased risk of adverse events and an increase in costs (Olumnese, 2006).

5.2. Artemisinin-based combination therapy

To counter the threat of resistance of *P. falciparum* to monotherapies, combinations of antimalarials are now recommended by WHO for the treatment of *P. falciparum* malaria. ART and its derivatives (artesunate, artemether, DHA) produce rapid clearance and rapid disappearance of symptoms. ART and its derivatives are also rapidly eliminated. When given in combination with rapidly elim-

inated compounds (tetracyclines, clindamycin) a 7-day course of treatment with an ART compound is required; but when given in combination with slowly eliminated antimalarials, a shorter course of treatment (3 days) are effective. Exposure of 3 days to ART will reduce the number of parasites, but complete clearance is dependent on the partner medicine.

The following ACTs are currently recommended by the WHO for uncomplicated malaria:

- Artemether–lumefantrineThe total recommended treatment is 2-dosages per day for 3 days containing 20 mg artemether and 120 mg lumefantrine per dosage. It is co-packed, which minimize the change of monotherapy occurring. A disadvantage is that it should be co-administered with fat. This is not always widely present in rural areas in Africa.
- Artesunate + amodiaquineThe total treatment is 1 dosage per day for 3 days, but the drug should be administered according to weight. The current dosage consists of 20 mg of artesunate and 153 mg of amodiaquine. Co-packaging is not possible, and therefore there is an increased change of resistance developing.
- Artesunate + mefloquineThe total recommended treatment is 1 dosage per day for 3 days of artesunate (50 mg) and mefloquine (250 mg) spread out over 2 or 3 days. Co-packed tablets are under development, which will decrease the risk of resistance.
- Artesunate + sulfadoxine–pyrimethamineThe total recommended treatment is artesunate (50 mg) once a day for 3 days, according to weight, and one dose of sulfadoxine (500 mg)–pyrimethamine (25 mg) on day 1. Because co-packaging is no option, there is an increased risk of resistance.

Severe malaria requires a fast acting antimalarial, since severe malaria can be lethal in hours. Two classes of drugs are currently available for the treatment of severe malaria: the cinchona alkaloids (quinine and quinidine) and ART derivatives. Chloroquine is not longer recommended, because of widespread resistance. Since artesunate is soluble in water and can be given intravenously or intramuscular this is the first-line treatment. ART is formulated for rectal administration, which is sometimes necessary in patients with severe malaria. Once the patient can tolerate oral therapy, treatment must be continued and completed with ACT or quinine + clindamycin.

5.3. Making ACTs available by home-based malaria management

Since a complete self-reliant treatment for malaria is not yet possible, due to the high recrudescence rates of ART from traditional tea and the recommendations from the WHO, the antimalarials should be brought to the communities in Africa. Treatment of malaria in Africa is challenged by inadequate health-care infrastructure. Health facilities are often resource-limited, and access to care may be limited by distance, fees, inadequate staffing, and lack of essential medicines. Costs provide a major barrier for many households. Thus, febrile illnesses are often treated at home, frequently with drugs purchased from shops. It is estimated that fewer than 20% of children with malaria in endemic areas are treated in formal health-care settings (Hopkins et al., 2007). To improve access to antimalarials, the WHO is promoting HMM as a major strategy for Africa. HMM promotes the distribution of antimalarials by trained members of the community. These members provide medication and education to primary caregivers, administration of antimalarials, and recognition of severe illness. The drugs provided by HMM are pre-packed, so monotherapy treatment is not a large concern and adherence will be increased. Artemether-lumefrantine (Coartem) is at the moment the only pre-packed ACT available which is easy to administer. The rest are either co-administered or as single individual drugs leading to difficult administration and adherence regimes. With co-administrated combination therapy, there is an increased risk of monotherapy, since both drugs are independently effective. Hopefully, the artesunate + mefloquine trial with pre-packaging will be successful, so that this ACT can also be used (Alin et al., 1996; Depoortere et al., 2004).

Table 6
Advantages and disadvantages of HMM.

Advantages HMM	Disadvantages HMM
Increased access to antimalarials	Higher costs
Better timing of treatment	Increase in demand
Pre-packaging: better adherence	Over-treatment, resulting in risk of resistance
Pre-packaging: decrease in monotherapy	Payment: disrupting social patterns
	Urban areas: less effective HMM

However, there are downsides to treatment at home (Table 6). Unnecessary over-treatment with antimalarials could promote drug resistance and is likely to have economic consequences. Also, over-treatment increases the chance of developing resistant strains. Whether ACTs can be used safely and effective at home is a major question. The current costs are US$ 1.6–3.4 billion per year, and cost-effectiveness of ACTs in HMM is uncertain. Trials to provide evidence for HMM treatment or community based treatment published trails were revealed. This showed mixed effects on morbidity and mortality. No published data are available yet on use of ACTs in HMM programs. Implementation of home-based or community based programs have been showed to be affordable with non-ACTs regimens. However, cost of deploying ACTs in HMM will be high. HMM can have additional functions besides providing in ACTs. HMM can improve the overall appropriate use of drugs. Inappropriate drug use is a major problem in malaria countries due to poverty, ignorance and lack of effective drug regulatory instances. Absence of regulatory instances has lead to importation of substandard and unregistered drugs, uncontrolled prices, smuggling, under-dosing, and shift to the informal health sector (Whitty et al., 2004). Recently it has also come to light that Artemisia species other than *A. annua* are being sold as treatment for malaria (Van der Kooy et al., 2008). This is also an emerging and serious problem for the effective treatment of malaria. Creation of awareness and information on ACTs should increase the use of ACTs in malaria. This service can be provided by the trained members of the HMM program (Mutabingwa, 2005).

5.4. Additional problems in treatment of malaria

Direct and indirect costs are the main reason why adopting ACTs in poor developing countries is difficult. ACTs cost more than 20 times than that of monotherapies. Since artemisinins are not widely available, the cost will remain high for years to come. Novartis Pharma, producer of ACTs, and the WHO have reduced the cost of artemether–lumefrantine to around US$ 0.9–1.4 for a treatment course of a child up to 7 years and around US$ 2.4 per adult dose. Despite this reduction, prices are still high. One full treatment course for a child takes 10% of the monthly income. On average, one child may have four malaria episodes a year and another four episodes that may be wrongly treated as malaria, leading to significant high costs. Local production of raw materials will reduce costs (Mutabingwa, 2005). Because the costs of ACTs are high and the supply is short, the available drugs should be used with care. Diagnosis plays a key role in appropriate drug use. The diagnosis of malaria is based on a clinical diagnosis supplemented by the detection of parasites in the blood. The clinical diagnosis alone

has a low specificity and in many countries parasitological diagnosis is unavailable. Besides drug (and thus cost) saving properties parasitological diagnosis have additional advantages:

- Improvement of patient care, because of greater certainty that the patient has malaria.
- Identification of parasite-negative patients, which will need different treatments.
- Prevention of unnecessary uses of antimalarials, and thereby slow-down the process of resistance from developing.

Two methods are used for parasitological diagnosis: light microscopy and RDTs (Bell et al., 2006). Light microscopy is relatively cheap and highly sensitive, when used by well-trained staff it can be used to specify and quantify the parasite. The price of RDTs was recently reduced, so that they are now also cost-effective in some settings. RDTs vary in sensitivity and specificity and they are vulnerable to high temperatures. Despite these disadvantages, RDTs make parasitological diagnosis possible. The choice between RTDs and microscopy depends on local circumstances including skills available, the usefulness of microscopy for other diseases in the area, and the case-load. Where the case-load is high, microscopy will be less expensive. However, most malaria patients are treated outside health services, but are treated according to HMM. Microscopy is generally not feasible in such circumstances, but RDTs may be. In stable high transmission areas malaria is the most common cause of fever in children under 5 years. Antimalarials should therefore be given to children with fever or a history of fever, thus based on a clinical diagnosis. In this group, the risk of not treating false negative (based on parasitological diagnosis) is too high. In children of 5 years and above, malaria becomes progressively less likely to be the cause of the fever. In these children, parasitological diagnosis will be beneficial. Since HMM is promoted by the WHO, RDTs are probably the best option in these areas. In areas where two or more species of malaria parasites are common, only a parasitological diagnosis will result in accurate treatment. Since artemisinins are effective against all species, this treatment will be sufficient and parasitological diagnosis will have preference. In epidemic areas the case-load is too high for parasitological diagnosis.

6. Discussion and conclusions

Malaria is a major problem in tropical countries with 500 million infections a year and approximately 1 million deaths annually. Because resistance has been reported for all classes of antimalarials, the pharmaceutical industry is searching for new antimalarials with different mechanisms of action. The latest and current treatment is treatment with ART and its derivatives. ART is isolated from the shrub *A. annua* which was used to treat fevers and chills 2000 years ago as part of TCM. The leaves of *A. annua* are harvested and dried, which contain ~1% of ART. The purified ART can also be converted in more potent derivatives such as artemether. Since ATCs are recommended by the WHO, the short acting artemisinins need to be combined with another longer acting antimalarial.

Experiments have shown that effective amounts of ART are present in traditional prepared tea from *A. annua*, although the amounts vary due to several reasons including differences in extraction, collection, quantification and harvesting methods and environmental influences. The tea is however only effective in reducing the total parasitemia count below detectable limits and to eliminate symptoms. Squeezing the leaves seems to have a positive effect on the extraction efficiency, and is therefore recommended. By actively squeezing the leaves, some of the residual water is released from the leaves, which may contain additional active ingredients. How the extraction can be maximized through addition of external compounds such as fats, is not described in literature. However, this is an interesting question and should be further investigated.

To control the amount of ART in each cup of tea, the amount of ART in the plant needs to be constant. This may be accomplished by using cultivars such as “Artemis”. However, as shown above, the amounts of extracted ART still vary due to several reasons. The content of ART in *A. annua* should be further investigated, so that the dose of ART can be controlled better. One possible way of achieving this aim is to produce ready-made tea bag containing a specific amount of dried leaves. This simple “device” can be used to control the amount of plant material used for a tea preparation which will make the use of balances and filters obsolete. The addition of specific lipophilic components to these teabags (e.g. coffee creamer, milk powder etc.) might also improve the extraction efficiency of ART by hot water.

The traditional tea is effective against *P. falciparum* malaria, but high recrudescence rates does occur mainly because ART does not kill all stages of the parasite, ART and its metabolites have short half lives and the amount of ART in the tea is too low. Recrudescence is a risk for resistance. On the other hand, it may be argued that the use of *A. annua* preparations for the treatment of fevers has occurred in China for 2000 years, and continues to occur, apparently without the emergence of resistance.

It might be that the combination of ingredients in the traditional tea have a synergistic action which prevents the parasite to mutate, and build up resistance. This should be actively investigated; if the former is indeed true, ART in traditional tea from *A. annua* can be used against *P. falciparum* malaria as a monotherapy. Currently, there is no evidence to support this theory and a complete self-reliance therapy for the treatment of malaria with ART is therefore not possible yet without further scientific research.

Since the availability of antimalarials in rural areas is a major problem, ideally antimalarials should be grown and produced locally. However, *A. annua* can grow under African conditions (Snow et al., 2001; Barnes and White, 2005; Tuteja, 2007), and therefore farmers should be motivated to increase the production of *A. annua* with assistance from agriculturalists to help them to produce high yielding cultivars. For the production of 200 million adult courses, 4300–20,000 ha of *A. annua* plantations are needed. African countries can also not provide all raw materials, since the extracted ART needs to be combined with the additional drug (to produce ACTs), which cannot be produced locally. To accomplish this, the WHO has developed a system called HMM. A trained member will provide the drugs to local shops in pre-packed formulation. The only pre-packed current formulation is Coartem, but tests are also under way to produce artesunate + mefloquine in a pre-packed formulation. To make HMM successful, more funds for malaria research and policy implementation are needed. Funding agencies which support research on malaria includes the Global Fund, Bill & Melinda Gates Foundation, USAID, DFID, WHO/TDR and MMV. These foundations should support the HMM and aim at an increase of self-reliance by: (controlled) production of raw materials, isolation of ART, synthesizing derivatives, and the production of pre-packed formulations of ACTs. This will result in an increased availability of antimalarials, and hopefully to a decrease in mortality of malaria in sub-Saharan countries. Unfortunately, the need for antimalarials is a problem of the past, present and future. Development of strategies providing antimalarials and implementing it will take years, and until implementation is completed malaria will be the number one killer in tropical countries.

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