The role of herbal products containing Artemisia annua in malaria treatment. A proposal for further research. Concept 01 Oct. 08

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INTRODUCTION

Around 1.5 million people die every year of malaria; every 30 seconds a child dies due to this preventable and curable disease. Over 90 % of malaria cases and the great majority of malaria deaths occur in sub-Saharan Africa. Most of the affordable antimalarial drugs have become ineffective because *Plasmodium falciparum* – the malarial parasite responsible for the most severe malaria cases and deaths - has developed resistance to them.
According to the World Health Organization (WHO) and other agencies, artemisinin based combination therapies (ACTs), which contain derivatives of artemisinin (extracted from the plant *Artemisia annua*), are the most promising anti-malaria drugs for tackling this problem.
It has been estimated that there are roughly 500 million episodes of clinical malaria per year, the majority of which should ideally be treated with an ACT. (1)
Supply does not yet meet this biologically-induced need. The production and supply chain needs to grow and significant public and private interventions are required to make an effective and affordable anti-malaria drug available to African patients. Financial capacity should follow this biological-induced need rather than the effective market demand. Much of the financial capacity is provided by subsidies. The problem with them at this stage is that they are provided by a third party and so complicate/change the normal exchange process that takes place in a conventional market.

At this moment, many African governments are planning to introduce ACTs for first line treatment of malaria. Artemisinin or its derivatives contribute for at least €0,83 (USD 1,14) to the price of an ACT. Introduction of ACT in countries where chloroquine, amodiaquine or sulfadoxine-pyrimethamine were the drugs of first choice will imply at the very least a ten-fold increase of the budget needed for antimalarial drugs. (prices from IDA, 2007) (a)

Prices of artemisinin and its derivatives, and therefore of ACTs, are not likely to drop significantly in the near future due to the high costs of purification and isolation of artemisinin from the Artemisia annua herb and the need for further derivatisation. The price of the herb itself contributes less than $0,05 to the price of a standard ACT. (Willemien Lommen, pers. comms)
It is generally anticipated that a synthetic ACT will be available within 10 years (1, conclusion 6)
At the moment, no forecast can be made about its retail price. It is highly doubtful that a vaccine against malaria will be available in the near future.

There is a risk that when funding of ACT comes to an end, that is when the capacity-building phase of introducing new therapies in developing countries is finished, governments in developing countries might face the problems concerning sustaining the availability and affordability of this newly introduced therapy. (2)
From a technical point of view, it has been possible for many years to cultivate sufficient amounts of *A.annua* to produce enough ACTs to cure all the malaria patients in the world.

The fact that ACTs are still not widely available in malaria endemic areas might strengthen governments in developing countries in their belief that depending on the availability and affordability of Western drugs might not be the best approach to the problem.
When ACT's are not available or affordable, malaria cases will be treated (if treated at all) with either the old and in many cases less effective drugs, or with herbal products.
Some scientists argue that uncontrolled use of herbal products alternatives when it concerns Artemisia annua preparations might increase the risk of artemisinin resistance development. Others argue that the use of herbal products decreases the risk of artemisinin resistance development. Absence of hard evidence on this issue results in a debate that is based on emotional or political considerations rather than on scientific facts.
Even in the absence of herbal-product alternatives, the high price of Artemisinin Combination Therapies may lead to the use of inadequate dosages, generating the same risk of resistance development. The high price of ACT's is at least partly responsible for the marketing of fake or substandard ACTs. (Dana Dalrymple, Agriculture, *Artemisia annua*, Artemisinin, ACTs and Malaria Control in Africa: The Interplay of Tradition, Science and Public Policy , working paper September 30th 2008)

If safe, effective and quality controlled antimalarial preparations could be produced by simple means and at low cost from locally grown Artemisia annua, such preparations may offer an additional tool for malaria control until prices of ACT have dropped to an affordable level. They would also strengthen the position of developing countries when negotiating the price of (bio) synthetic artemisinin should it become available, and give developing countries the choice between importing medication or producing them.

Aqueous extracts of Artemisia annua, powdered whole-leaf tablets of Artemisia Annua, and organic solvent extracts of Artemisia annua are already being used as antimalarial preparations. However, reliable data on efficacy and safety of such preparations are extremely scarce, preventing a responsible consideration of their potential benefits and risks in malaria control.

It is argued that the use of Artemisinin-based tea, whole leaf powdered tablets or organic solvent extracts as a malaria treatment is not an option for mainstreaming into conventional health systems. They cannot be registered as a conventional drug, and therefore they cannot be prescribed, because of their non-standardized and variable composition.(1)

Recently, the WHO published guidelines upon information needed to support clinical trials with herbal products (3). If cheap, safe, effective and quality controlled preparations of AA could be registered according to WHO guidelines as a herbal product, policy makers could decide to advise that these herbal products *should* be mainstreamed into conventional health systems.

The fast-growing herb *A. annua* can be cultivated with relative ease in developing countries.(4) The artemisinin content of wild *A. annua* L. has been described to vary between 0.02% and 1.1% of the dry weight, depending on plant source and cultivation conditions. Yields of 1.4% of the dry weight can be obtained from a hybrid called *Artemis* that has been developed for commercial artemisinin production.(5)

Aqueous extracts:

Safety:
The current pharmacopoeia of the People's Republic of China officially lists the dried herb of *A. annua* as a remedy for fever and malaria.(6) The daily dose is specified as 4.5 to 9 grams of dried herb to be prepared as a tea infusion with boiling water.
25-30 grams of the dried herb boiled for 30 minutes renders an analgesic and antipyretic decoction to be taken once daily for seven days. (7)
It has as so been used for at least 2000 years without the report of serious adverse events and therefore can be considered as a safe and established treatment.

Pharmacokinetics:

Artemisinin itself is poorly soluble in water, but appears to be solubilizedin the presence of other plant constituents with amphiphilicproperties (e.g., flavonoids or saponins).(8)
The artemisinin concentration in such traditional tea preparations varies widely and the claimed maximum content of 94,5 mg artemisinin per liter tea (8) using 9 grams of dried leaves (extraction efficiency 76%) has not been reproduced. The failure to reproduce this extraction efficiency has been ascribed to differences in analytical procedures determining the artimisinin concentration. (9) However it must be noted that these different analytical procedures did not result in the finding of different artemisinin concentrations in the dried leaves of the plant itself. (8,9,10).
Pure Artemisinin is absorbed only partly in the gut, resulting in low bio-availabilty of Artemsinin and dihydroxy-artemisinin. It was argued that bio-availability of artemisinin could be increased by other constituents of the aqueous extract of AA. Although clinically relevant plasma concentrations of artemisinin can be achieved taking the traditional tea preparation, the bioavailability of artemisinin from aqueous extracts of Artemisia is similar to the bioavailability of pure artemisinin (8).

Efficacy:

Mueller et al did a study using 9 gram of dried AA prepared as a tea reportedly containing 94,5 mg artemisinin /day for seven days . Cure rates were 70 % on day 7, on day 35 the cure rate dropped to 30 % as a result of high recrudescence (11,28). Higher efficacy and lower recrudescence rates have been reported using AA tea in combination with amodiaquine or SP but no studies were performed with these combinations.(www.anamed.net)

Whole-leaf powdered tablets.(b)

Safety:
In China, Lupus Erythematosus and Oral mucosa lichen planus are treated by daily ingestion of 36-54 grams fine ground dried AA leave for 1-3 months. (3)No serious adverse events have been reported to my knowledge. A recent study in Kenya showed only minor adverse events using Whole-leaf powdered tablets for the treatment of malaria.(12)

The artemisinin content in the dried leaves of AA can be expected to be 0,8%-1,4%. Therefore, 7,1-12,5 gram of dried leaves of AA will contain 100 mg artemisinin.

Efficacy:

Recently, Prof. Hassan Ali conducted a study at the Kenya Medical Research Institute with remarkable results.(12)

It was an open-label, dose-rising, non-randomised single centre study for the efficacy, safety and tolerance of increasing doses of A. annua tablets in informed consenting individuals with uncomplicated malaria. The medicine was supplied as 500mg tablets prepared from dried crushed finely powdered whole leaf of the herb, each containing approximately 3.74 mg of artemisinin. The drug was administered orally in progressively increasing doses on four cohorts (C1, C2, C3, C4) as follows (level of artemisinin shown in brackets).
C1: 2 tablets (7.4mg) twice a day for day 1; 1 (3.7mg) tablet twice daily for the next 5 days.
C2: 3 tablets (11.1mg) twice a day for day 1; 2 tablets (7.4mg) twice daily for the next 5 days.
C3: 4 tablets (14.8mg) twice a day for day 1; 3 tablets (11.1mg) twice daily for the next 5 days.
C4: 5 tablets (18.5mg) twice a day for day 1; 4 tablets (14.8mg) twice daily for the next 5 days.

In Cohort 1, Eleven (91.66 per cent) of the 12 patients reported relief of clinical symptoms and signs by the third day of treatment. Of these 12 patients, 83.33 per cent (10) had no malaria parasites by day six.
Eleven patients (91.6 per cent) had no parasitaemia or clinical complaints by day seven.
On day 14, 10 of the patients (83.33 per cent) had negative blood smears. And on day 28, nine (75 per cent) of the patients had negative blood smears on Giemsa staining for malaria parasites.

Pharmacokinetics:
No studies on pharmacokinetics are available to my knowledge

Practical issues:

Whole-leaf powdered tablets can be made with relative ease in developing countries. Determination of artemisinin content in these tablets can be done also with relative ease using a recently developed Thin Layer Chromatography (TLC) method which is within 1 % accuracy of the High Performance Liquid Chromatography (HPLC) method which is considered to be the golden standard.(13) Compared to the inter-individual variability in bio-availability of artemisinin this inaccuracy can be neglected.

Artemisinin levels in dried leaves are known to drop rapidly when improperly stored under tropical circumstances. (12) In proper storage conditions, the artemisinin is present in almost whole amounts even after one year of storage (16). I could not find data on the shelve-life of AA whole-leave powdered tablets but it seems logical that these studies are being or have been performed at the Kenya Medical Research Institute. If not, shelve-life studies can be performed with relative ease using the TLC method.

There is a little technical problem with the herbal pills of AA: The dried leaves ought to be cleaned to make sure there are no bacteria etc on them, but the usual cleaning processes diminish the artemisin content - so there is the need to experiment on ways of solving this problem, at the moment the knowledge how to do this is only limited to some very few companies who do not share their knowhow. Another problem is that leaves with a very high artemisinin content are needed which are usually more expensive, so savings as compared to

extracted Artemisinin might not be quite as high as one would expect. (Von Freyhold, pers.comm)

Obviously ingestion of 7,1-12,5 grams of a herbal product twice daily is a bit unpractical, but so is having no medication at all.

Organic solvent extraction

A recent comparative assessment of technologies for extraction of Artemisinin compared extraction with Ethanol, Hexane/Petroleum ether, Supercritical CO2, Ionic Liquids and Hydrofluorocarbons. These extraction procedures are used as the first step in the process of isolation and purification of artemisinin. (14)
The benign nature of ethanol and its widespread availability from renewable feed stocks combined with its simple technology makes ethanol extraction the only organic solvent extraction method that is useful if you want to use the concentrated extract as a drug/herbal product. The fact that ethanol extraction is not selective for artemisinin is a disadvantage when the purpose of extraction is to produce pure artemisinin. In the light of the presence of other constituents in Artemisia annua that contribute to its antiplasmodic activity, it may well prove to be a great advantage when the extract itself is used as a drug/herbal product.

Safety

Essential oils are considered to be the most important contents of AA when safety is concerned. Essential oils are present to a certain extent in an ethanolic extract. Perazzo performed a study on rats with an ethanolic extract of AA and with the essential oils from AA. (15) The LD 50 of the essential oils was 790 mg/kg. This corresponds with 61 gram/kg bodyweight of dried AA leaves. The maximum dose of ethanol extract used in this study was 2 gram/kg bodyweight corresponding with 17,6 gram/kg of the dried leaves.
Yao De did a study on mice and on humans with an ethanolic extract of AA. (17) (c)
The LD50 of an ethanolic extract of AA in mice infected with plasmodium Berghei was equivalent to 162.5 +/- 10.1 gram dried leaves/kg.
An equivalence of 73 gram of the dried herb (total artemisinin content 230 mg) was given to humans in 3 days and 128 gram (total artemisinin content 402 mg) was given in 6 days with no adverse events reported. The artemisinin content of the leafs used in this study was 0,3%.

The minimum artemisinin content in the dried leaves of AA can be expected to be 0,8%-1,4 %. The extraction efficiency of ethanolic extraction will be 80-95 %. (14,27)
100 mg artemisinin in an ethanolic extract therefore will be derived from 7,9-15,6 gram of dried leaves. This is 0,3 gram or less/kg bodyweight. 600 mg artemisinin will be extracted from 47-94 gram dried AA leaves, corresponding with 1,8 gram or less/kg bodyweight.

It must be noted that ethanol is only used to facilitate extraction. All ethanol will be removed from the final concentrated extract. All ingredients in a concentrated ethanolic extract are also present in at least the same concentration in the dried leaves from which the extract is made.

Efficacy:

Efficacy of an ethanolic extract of AA depends on the final formulation. To my knowledge, only formulation as a tablet and formulation as gelatin capsulae containing the extract dissolved in oil have been tested, the gelatin capsulae being 3.9 times more potent than the tablet formulation, probably because of the presence of oil.(17)

Gelatin capsulae containing the ethanolic extract of AA dissolved in oil were tested on humans infected with plasmodium vivax. (17)
An equivalence of 73 gram of the dried herb (total artemisinin content 230 mg) was given to humans in 3 days and 128 gram (total artemisinin content 402 mg) was given in 6 days resulting in complete clearance of parasites in both groups upon completion of the treatment.
Recrudescence rates were 33 % in the 3 day treatment group, 12,5 % in the 6 day treatment group.
An equivalence of 83 gram (total artemsinin content 260 mg) of the dried herb combined with pimaquine was given to patients in 6 days. In this group, no recrudescence was seen.
A 3 day treatment of ethanol extract combined with primaquine has not been tested or has not been published.
Recrudescence rates where lower when the drug was given in multiple daily doses.

Practical issues:

Ethanol extraction of AA can be done in developing countries. (14) Artemisinin extraction rates of 95 % can be obtained using ethanol. Ethanol extraction is more cost-effective than hexane extraction (27). The crude, dried ethanolic extract of AA is a dark green, sticky substance containing roughly 16 % artemisinin.

Semi-refining of the crude ethanolic extract of AA with the polar HFC R-134a, which removes polar impurities such as sugars, renders a dry powder with an artemisinin content between 60-80%. (26) This has great advantages concerning handling and reduces the volume of the active compound. Since the mechanism of the increased potency of ethanolic extracts of AA dissolved in oil is not yet understood, it remains uncertain if the proces of semi-refining reduces the potency of the extract.

Other advantages of ethanolic extracts and semi-refined ethanolic extracts of AA over derivatives of artemisinin, besides the reduction in costs, are that they require relative low-tech facilities and do not have the problems associated with the disposal of chemicals used in further purification and derivatisation.

Shelf life of Gelatin capsulae containing the ethanolic extract of AA dissolved in oil: artemisinin content diminished with 10.7% and antiplasmodial potency in mice infected with Plasmodium Berghei was lowered by 12% after 3 months of storage at 37% (accelerated storage method), corresponding with 2 years storage at normal temperature (17)

Costs:
Artemisinin in the crude ethanolic extract will cost roughly $ 220/kg. Artemisinin as a purified substance will cost roughly a minimum of $290/kg. Artesunate will cost minimal $520/kg. (other quotes on the price of artesunate are $840/kg and $ 1000/kg)

Artesunate is 5 times more potent then artemisinin, therefore artesunate is relatively 2.5 times cheaper then artemisinin. If artemisinin in the form of an ethanolic extract dissolved in oil will prove to be 4 times more potent then artemisinin as an isolated compound, costs will be less than half the price of artesunate.
Capital and running costs can be reduced by getting a tax-exemption for the ethanol used and by reducing the requirements for heat to evaporate large amounts of EtOH-H2O azeotrope after each extraction cycle. Heating costs can be reduced under tropical circumstances by pre-heating the EtOH-H2O azeotrope using direct solar energy.

Discussion:

Initial research suggests that the plant Artemisia annua has a more potent antiplasmodial activity than can be explained by its artemisinin content only (8,11,12,16,17,20-24) (d). It is unclear whether this should be ascribed to increased bioavailability, presence of precursors of artemisinin, which may act as prodrugs, or presence of other constituents with antiplasmodial activity in Artemisia annua. Prof Hassan Ali observes that apart from artemisinin, there are another 13 closely related compounds that have been isolated from the herb, some with probable synergistic effects on artemisinin (12). It was therefore argued that the whole-leaf powdered tablets, aqueous extracts or ethanolic extracts of Artemisia annua could be regarded as Artemisinin Combination Therapies. (11,12,17). However, clinical trials with such herbal products resulted in high recrudescence rates. An exception is the study at KEMRI where powdered whole-leaf tablets where used resulting in recrudescence rates comparable to artemisinin monotherapy but at markedly lower doses, but these results have not been published officially yet.The number of patients in this study was small however, and the methodology unclear

Herbal products containing AA with lower artemisinin content than conventional drugs seem to have a clinical effect and can be considered as safe. As with artemisinin and artesunate monotherapy, recrudescence rates are relatively high. These recrudescence rates must be interpreted with some care: A recent study showed that half of the suspected cases of recrudescense where in fact re-infections (Kefas Mugittu et al) . A study with an ethanolic extract of AA combined with primaquine resulted in no recrudescence This was in vivax malaria (17). For reasons of risk of artemisinin resistance development herbal products containing AA should be combined where possible with other anti-malarial drugs such as amodiaquine, sulfadoxine/pyrimethamine, mefloquine or other herbal anti-malarials.

Although herbal products containing AA seem to have a clinical effect, the risk of (and the fear for) the development of artemisinin resistance calls for a rather unorthodox approach to further clinical research: rather than to look for the minimal safe and effective dosage of a herbal product containing AA combined with other antimalarial medication, the minimal dosage of a herbal product containing AA with referral to artemisinin content should be determined on theoretical grounds to minimise the risk of resistance development to artemisinin.

It seems that the therapeutic indices of artesunate and of herbal products containing AA with referral to artemisinin content are quite similar (8,11,12,17). I therefore propose to investigate the safety and efficacy of Artemisinin Combination Therapies consisting of herbal products

containing artemisinin at a dosage equal to the dosage of artesunate (600 mg in a standard adult treatment regimen), combined with other antimalarial medications at dosages normally combined with artesunate.

Artemisia Tea

Since aqueous artemisinin extraction efficiency diminishes rapidly upon increasing the amount of grams dried leaves added to a litre of water (18), ingestion of 600 mg artemisinin will most likely only be achieved by either ingesting unpractical amounts of tea or by extracting very high amounts of dried leaves. The artemisinin content in stored Aa leaves diminishes quickly under tropical circumstances if not stored properly(12) Although quality controlled pre-packed Artemisia tea bags could be made and stored properly, the traditional tea preparation still has to be made freshly every day. Artemisinin concentrations in the tea drop rapidly when this is not done correctly. (8)

For these reasons it seems that the tea-approach will always be subject to criticism concerning efficacy and risk for developing artemisinin resistance.

The main benefit of Artemisia tea is that it can be made available sustainably to those communities not reached by the modern drug distribution system. (Merlin Willcox, RITAM, pers.comm). More research is needed on actual artemisinin content in AA tea since the findings of Rath have not been reproduced. Since AA tea is becoming widely praticed, it is important to experiment with different preparations that might reduce the risk of artemisinin resistance development. Yao-De Wan states that AA tea prepared with milk instead of water is more effective, probably because the fat-content of the milk. This would be in line with his findings that the pressence of oil increases the potency of ethanolic extracts of AA. Milk is not widely available to most people in sub- sahara Africa, so one might want to experiment with oil instead.

Ethanolic Extracts and Whole Leaf Powdered Tablets of AA

For reasons of quality-control as well as for practical reasons, whole-leaf powdered tablets and ethanolic extracts are the most likely candidates to render an inexpensive, safe and effective herbal product when used in combination with other antimalarial medication as part of an ACT.

It is a well known phenomenon that plasma concentration of artemisinin reduces rapidly in the course of the treatment if given in monotherapy. The most logical explanation is an increased metabolic capacity due to autoinduction (19). The equivalent studies have not been performed, to our knowledge, for the herbal products. The same effect may not occur if other constituents prevent autoinduction. In all studies (11,12,17) with herbal products containing AA parasite counts dropped rapidly within 24 hours and in most studies parasites could not be detected after 3 days treatment (12,17). Therefore it seems logical to investigate a 3 day-regimen with herbal products containing AA as part of an ACT . Obviously, this will also enhance patient compliance. However, in studies with herbal products containing AA it was also demonstrated that recrudescence rates diminished upon prolongation of the treatment. A minimum duration of treatment of 7 days seems optimal when using the herbal product containing AA as a monotherapy. (11,12,17) Therefore, it cannot be entirely ruled out that a 6-7 day regimen with a herbal product containing AA as part of an ACT will prove to be more effective than a 3 day

regimen. However, the disadvantages of such a long treatment seem greater then the financial advantages of a herbal product.

The WHO states that phase 1 studies in normal volunteers are generally unnecessary for herbal traditional medicines.
The substantial prior human use of traditional dose regimens of herbal medicines generally conveys reasonable confidence that these regimens can safely be administered to small numbers of carefully monitored clinical subjects in phase 2 trials. (3)

I propose to investigate the following questions:

1 Can increased potency of (semi-refined) ethanolic extracts of AA dissolved in oil be explained by the pressence of oil, resulting in increased bioavailability of artemisinin?

2 Can therapeutic plasmalevels of artemisinin be obtained using (semi-refined) ethanolic extracts of AA as the source of artemisinin in an ACT?

3 Are (semi-refined) ethanolic extracts of AA dissolved in oil safe and well tolerated when combined with amodiaquine?

4 Can increased potency of (semi-refined) ethanolic extracts of AA dissolved in oil be explained by the pressence of other constituents that prevent autoinduction?

Obviously, encouraging answers to these questions will lead to formal clinical tests regarding efficacy of the product in malaria patients.

(a) A 3 day ACT consisting of 100 mg artesunate twice daily combined with three tablets of amodiaquine 200 mg base daily is the cheapest option (WHO Essential Drug guidelines). IDA is among the cheapest organisations to provide those drugs. It is a non-profit company and is recommended by WHO.
IDA charges 13,90 euro for 100 tablets of artesunate 100 mg, that is 0,83 euro for 6 tablets,a standard adult treatment. 1000 Amodiaquine 200 mg base tablets cost 8,55 euro. That is less than euro 0,08 for a standard adult treatment. Total cost of the ACT (6 tablets artesunate 100 mg and 9 tablets amodiaquine 200 mg base) is 0,91 euro, artesunate contributes for 91 % to this price. It is estimated that an ACT of artesunate/amodiaquine could be produced for $ 0,84 (Von Freyhold, pers.comm)

(b) Whole leaf refers to the way the tablets are made: The whole leaf of the Artemisia plant is dried, crushed, mixed and than pressed to form tablets. In fact you could just as well eat the dried leaf itself, but mixing te crushed leaves provides a way to determine the exact amount of artemisinin present. Artemisinin content in dried leaves varies widely, mixing the leaves is the only way to perform quality control of an active ingredient, an essential issue to obtain registration as a herbal product.

(c) Prof. Yao-De Wan uses the term: concentrated non aqueous extract. He has confirmed that he used ethanol as the solvent (pers. comm.)

(d) 'If beneficial effects (of Artemisia tea) are seen, this is not due to Artemisinin alone but also to other ingredients present in the tea' (10,20,21). Jansen (10) acknowledges that the effectiveness of the tea is partly due to other ingredients, as the dose of artemisinin is too low to account for the observed effect. This is confirmed by in vivo experiments in mice which showed that A. annua infusion reduces parasitaemia by 50% at day 4, compared to the equivalent dose of pure artemisinin, which was not significantly more effective than placebo (Plaizier-Vercammen, unpublished). Evidence is accumulating for the potentiating role of the polymethoxyflavones chrysosplenol-D, eupatin, cirsillineol, casticin, chrysoplenetin and artemetin (20,22,23)
These other ingredients also merit further research, to see whether their presence hinders the development of parasite resistance compared to pure artemisinin.(16)
For decades scientists have condemned valerian tincture because no single, effective substance could be isolated. The conflict was resolved by the acknowledgement that valerian tincture is only effective because of the synergy of all the various constituents, and to isolate one ingredient makes no sense. Many independent scientists confirm that this is also true for artemisia (tea) (24).

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