Journal of Ethnopharmacology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Review

The complexity of medicinal plants: The traditional *Artemisia annua* formulation, current status and future perspectives

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ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form
1 August 2013
Accepted 6 August 2013

Keywords:
Artemisia annua
Artemisinin
Cancer
HIV
Malaria
Tea infusion

ABSTRACT

Ethnopharmacological relevance: Artemisia annua has a long tradition of use for the treatment of intermittent fevers which we now relate to malarial infections. The active principle artemisinin has been isolated from *Artemisia annua* and today forms the backbone of the global fight against malaria. The traditionally prepared *Artemisia annua* formulation is however still being used on a global scale for the treatment of malaria, and it is claimed that its action is superior to the single purified drug. *Artemisia annua* is therefore on the forefront of the heated debate between the single drug–single target approach of western based medicine and the holistic approach of traditional medicinal systems. This review aims to highlight the complexities we face in the general study of medicinal plants at the hand of three levels of complexity. These levels consist of (a) the chemistry of the medicinal plant, (b) the influence of the preparation method on the chemistry of the final formulation and (c) the influence of metabolism on the chemistry of the formulation. We also aim to provide an up-to-date report on all scientific work that has been conducted and published in English on the traditional formulation of *Artemisia annua.*

Materials and methods: All English scientific literatures published until the first quarter of 2013 were retrieved from well-known scientific databases (Scifinder scholar, Web of Science, PubMed, Google scholar) and Non-governmental organisations active in this field were consulted. A draft version of this manuscript was sent to the African office of the World Health Organisation (WHO), and to the Non-governmental organisations "Action Médicine Naturelle" (ANAMED) and "Iwerliewen fir bedreete Volleker – Réseau belgo-luxembourgeois pour la valorisation des herbes médicinales" (IFBV-BELHERB) for comments.

Results: Very little scientific work has been conducted on the *Artemisia annua* formulation. The available literature contains many discrepancies which are unfortunately selectively being used by the two different sides in this debate to further their arguments. On one side of the argument we have the low content of artemisinin in *Artemisia annua*, the low bioavailability of artemisinin when the traditional formulation is administered and the high levels of recrudescence, which are being emphasised, while on the other side the possible role of synergism and prodrugs are being highlighted. This review reports that there are still too many gaps in our existing knowledge to provide conclusive evidence for either of the two sides of the argument.

Conclusions: Much more research is needed into *Artemisia annua* formulations. We stand to gain invaluable knowledge into how traditional medicinal plant works, discover the identities of new active compounds (which can be used against other diseases such as HIV, diarrhoea, and cancer) and possibly bring both sides of this debate closer together.

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http://dx.doi.org/10.1016/j.jep.2013.08.021

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1. Introduction

1.1. Background on the traditional use of Artemisia annua and its implications

Artemisia annua L. (Asteraceae) has been used throughout the ages to treat various ailments, specifically those related to the treatment and prevention of fevers which we now relate to malarial infections. Malaria is a vector-borne infectious disease caused by *Plasmodia* parasites, of which *Plasmodium falciparum* is the most infectious and lethal. *Plasmodium falciparum* infects over 500 million people each year, causing the deaths of more than 1.2 million people and also causes tremendous economic losses in the most affected countries (De Ridder et al., 2008; Murray et al., 2012). The majority of deaths can be attributed to specific high risk groups of which children under five are the most at risk (De Ridder et al., 2008, Willcox et al., 2011).

In ancient China, symptoms relating to *Plasmodium falciparum* infections were effectively treated with the *Artemisia* plant. This history of use is however quite ambiguous as the *Artemisia* plant was referred to as *qing hao* (possibly *Artemisia apiacea* Hance. (Asteraceae)) and *cao hao* (possibly *Artemisia annua*), without sufficient differentiation given between the two species. The differentiation was only provided in the year 1086 when the scholar Shen Gua (1031–1095) noted the differences between *Artemisia annua* and *Artemisia apiacea* (Hsu, 2006, 2010). The ancient preparation method of *Artemisia annua* and *Artemisia apiacea* involved soaking the fresh whole plant in water overnight followed by wringing it out and ingesting the resulting juice or emulsion. A second preparation method has also been discovered in an ancient manuscript and occurs repeatedly in many formulations during the Tang dynasty (618–907). This method involved soaking the entire plant in urine, while it was noted that this preparation method probably decreased contamination with harmful bacteria and increased the extraction efficiency of the active components (Hsu, 2006, 2010). A third method is described in which the plant material is baked on a hot plate until slightly scorched. None of these ancient preparation methods and its influence on the chemical makeup of the final formulation has yet been studied in any detail.

The present day use of *Artemisia annua* to treat malaria is to prepare and consume a tea infusion of the dried leaves of only *Artemisia annua*. The main contradiction between the books from antiquity and the present day use is that historically *Artemisia apiacea* was preferred over the use of *Artemisia annua*. Other discrepancies include that water or urine was used to prepare the formulation at room temperature using the fresh whole plant as opposed to the current practice of preparing a tea infusion of only *Artemisia annua*. These differences in preparation methods can potentially have a big influence on the chemistry of the final formulation. Two excellent reviews on the traditional use of *Artemisia annua* and *Artemisia apiacea* including translations from the ancient Chinese texts were published by Hsu (2006, 2010).

Currently there is renewed interest in using traditional Chinese medicine (Heide, 2006) and future research into the use of *Artemisia annua* and *Artemisia apiacea* can have important implications for antimalarial treatments in developing countries. Especially where other antimalarials may not be readily accessible (Hsu, 2006) or where the high cost of these drugs makes it practically inaccessible to many. Another important factor that is often overlooked is that people in many developing countries do not trust Western based drugs. The reason for this is debatable and the extent of this predicament will be very difficult to measure, however this aspect should not be underestimated. A good example of this lack of trust in Western drugs or where the high cost of these drugs are making it inaccessible, is the recent approval of a prophylactic malaria treatment by the Ugandan government consisting of *Artemisia annua*, lemongrass extracts and ground kernels of avocado, called Artavol. In a recently broadcasted British Broadcasting Cooperation radio interview this was hailed as one of Africa's scientific breakthroughs, together with an electric car, resulting from increased spending on scientific research.

Due to this renewed interest and the growing use of medicinal plants, it is of great importance to study commonly used medicinal plants in detail. The WHO is aware of these issues and intermittently publishes guidelines on the use of specific medicinal plants. In the case of *Artemisia annua* a relatively simplistic view is followed by the WHO and the scientific community active in studying this medicinal plant. In order to reach the prescribed doses of the main active compound artemisinin, they have concluded that Artemisinin Combination Therapies (ACTs) are the best way to approach the treatment of malaria (Mueller et al., 2004; Weathers et al., 2011; WHO, 2012) and that the traditional use of *Artemisia annua* should be discouraged. The ACT treatment regime makes use of artemisinin derivatives in combination with other slower acting antimalarials.

There are however two conflicting opinions. On one side the majority believes that using *Artemisia annua* tea infusions (or the whole plant treatment) will lead *Plasmodium falciparum* to become resistant to artemisinin. This is based on the low levels of artemisinin in *Artemisia annua* leading to difficulties in quality control and standardisation, the low extraction efficiency of water, the low bioavailability of artemisinin if taken in the form of an infusion and the high recrudescence rates in patients using the infusion. The minority view claims that artemisinin is not the only active compound in the infusion and that synergism plays an important role in the overall efficacy. The possible presence of

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pro-drugs in *Artemisia annua* also plays a potentially integral part in the treatment. Other compounds in *Artemisia annua* might also aid in increasing the bioavailability of artemisinin although this effect is much more prominent if taking the dried leaves as opposed to a tea infusion. The production and storage of artemisinin *in planta* is also cost effective and it can be locally cultivated to increase availability. Lubbe et al. (2012) has shown that the artemisinin content in the *Artemisia annua* leaves is stable for a prolonged time. Using medicinal plants also fits well into the current health care systems in developing countries and it is suggested that the *Artemisia annua* infusions and decoctions have been used for centuries without resistance developing while resistance has only recently emerged in Thailand where pure artemisinin has been used as a mono therapy for a number of years before the introduction of ACTs (Phyo et al., 2012). Counterfeit and/or substandard artemisinin is also an escalating problem and has caused numerous infected people to be treated with sub-optimal doses of artemisinin (Newton et al., 2011) which might have led to resistance developing.

This clash between western based drugs (e.g. artemisinin) and traditional medicines (medicinal plants e.g. *Artemisia annua*) has culminated in the publication of a Position Statement by the WHO on the use of *Artemisia annua* for the treatment of malaria. The main recommendation in this statement was not to use *Artemisia annua* in any form including a tea infusion due to the low natural abundance of artemisinin (0.01–1.34%) in *Artemisia annua* (Liu et al., 2006; WHO, 2012). Importantly the WHO report also states that "extensive fundamental and clinical research would be required to demonstrate that non-pharmaceutical forms of *Artemisia annua*, including tea bag, are safe and effective to treat malaria and that their dissemination would not promote the development of artemisinin-resistant parasites" (WHO, 2012). This position statement has prompted us to gather all available scientific literature (in English) published on the traditional *Artemisia annua* formulation in order to

(a) summarise the available scientific information regarding the traditional formulation of *Artemisia annua* in one document;
(b) highlight the discrepancies found in the available literature; and
(c) recommend and streamline future research efforts on the traditional use of the *Artemisia annua* formulation for the treatment of disease.

It is not our intention to advocate the use of one treatment method over another but merely to present current scientific data in order to make informed decisions and to streamline future research efforts into the use of the *Artemisia annua* formulation. In the next section we will shortly describe some of the complexities we face in our general aim to understand how medicinal plants work. This will be followed by summarising the available chemical, bioactivity and pharmacological data published on the traditional *Artemisia annua* formulations until the first quarter of 2013. In Section 6 we will summarise the current research results and then focus on the future perspectives for research into *Artemisia annua*.

2. Complexity of traditional medicinal plants

Plants and plant derived products have been used as medicine for millennia, where the fate of societies and the outcome of wars has been decided on the provision of adequate treatment of diseases, especially infectious diseases (De Ridder et al., 2008). We are not referring to a thousand years ago, but only to 85 years ago, before the important discovery of antibiotics in the 1920s. This remarkable discovery did however have one negative impact, in that it steered us into adopting a single drug–single target

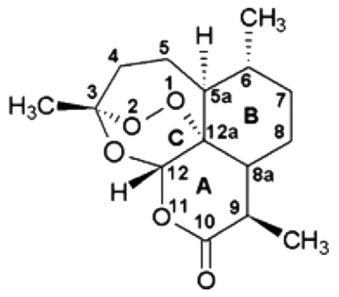

Fig. 1. The chemical structure of artemisinin indicating the seven stereogenic centres present in this molecule.

mindset. Modern scientific research with advanced analytical capabilities into the functioning of medicinal plants has only taken place for a number of decades but due to our single drug–single target mindset it has mostly been sidelined, while the focus shifted to the identification of single active compounds. It is an accepted fact that traditionally used medicinal plants is chemically complex, making it difficult to determine their efficacy, toxicity or mechanism of action. This complexity is unfortunately also being used to support the continued use of some medicinal plants without any scientific evidence for their efficacy and thereby causing damage to this research field.

Another issue facing the use of medicinal plants is that a long history of use is normally considered to be an evidence for their efficacy and non-toxicity. However, we must question the unqualified basis of this as a plant used a thousand years ago might have had a completely different chemical profile than the same plants (or species) today, due to differing climatic conditions and other environmental influences. With the advent of taxonomy by Linnaeus in the 18th century and by using divergent taxonomy many species have been named and renamed over the years. This can complicate the correct identification of a medicinal plant, which is key for the successful scientific investigation. Therefore a specific plant might have worked a thousand years ago but that does not necessarily mean it will work today, due to a different chemical makeup, or because the knowledge was lost due to the species being given a different name in the modern age. A medicinal plant might also have worked intermittently over the years due to a fluctuating chemical profile based on the climate of the day and might therefore have withstood the test of time. Even a well-known medicinal plant such as *Artemisia annua* which has been used 2000 years ago was "forgotten" in the middle-ages until it was rediscovered again in the 1970s, although some scholars suggest that it was not *Artemisia annua* that was used 2000 years ago but *Artemisia apiacea* (Hsu, 2006). Nevertheless, the rediscovery of this medicinal plant has probably yielded one of the most valuable compounds from a medicinal plant to date, artemisinin.

The complexity of traditional medicines consists mainly of three levels, namely the plant chemistry, the chemistry involved in the preparation method to yield the final formulation and finally the chemistry and pharmacology involved after administration of the formulation, hence its biological activity, bioavailability and the overall adsorption, distribution, metabolism, excretion and toxicity properties of each important molecule. In this review we will report on what is currently known on all these three levels focussing on the traditional formulation of *Artemisia annua*.

2.1. Level 1. Complexity of the plant chemistry

If we take a deeper look at *Artemisia annua*, we can start to appreciate the complexity of the chemistry involved but also the potential for discovering some remarkable attributes including new lead compounds for the treatment of different diseases. From this

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plant a single active compound, artemisinin, has been identified which is currently being used in a derivatised form for the treatment of *Plasmodium falciparum* infections. Artemisinin has seven stereogenic centres which mean that 128 stereoisomers are possible (Fig. 1).

Only one stereoisomer is known, epiartemisinin (C9 isomer) which is remarkably less active against *Plasmodium berghei* and *Plasmodium yoelii* than artemisinin, but has not yet been tested for activity against *Plasmodium falciparum* or other diseases such as cancer (Jefford et al., 2000). Drying the plant material or the purified artemisinin leads to crystallisation of artemisinin into different crystal types or polymorphs (depending on the drying method and solvents involved), each type having its own aqueous solubility and dissolution rate. For artemisinin two polymorphs are known, the triclinic form (aqueous solubility=48 mg/L, dissolution rate=4 h) and the orthorhombic form (aqueous solubility=20 mg/L, dissolution rate =18 h) (Chan et al., 1997). The crystal type can therefore have a profound influence on the bioavailability and when the maximum plasma concentration (C_{max}) is reached after administration. It is also known that any given plant contains thousands of different secondary metabolites of which many will have possible stereoisomeric and/or polymorphic forms. Primary metabolites such as (sulphated) polysaccharides might also have a role to play in the efficacy or the synergistic effects ascribed to a traditional medicine. Taking all of these abovementioned factors together, it paints a highly complex picture. There is however another layer of complexity to add.

2.2. Level 2: Chemical influence of the preparation method

We all know that a cooked potato tastes different than a raw potato, indicating that the preparation method has changed the taste and therefore the chemistry. But as scientists we tend to focus only on what people used as medicine (e.g. *Artemisia annua*) and far less on how they used it (e.g. tea infusion, food, smoked). Currently, the preparation of a tea infusion of *Artemisia annua* is prescribed. During preparation of the tea infusion we use “super clean” deionised water in the laboratory (acidic pH, low electrical conductivity) whilst people using the tea as a treatment use any locally available “dirty” water (e.g. tap, borehole, river, rain water). If we keep in mind that the mechanism of action of artemisinin is thought to occur via iron mediated activation (De Ridder et al., 2008), it is quite possible that metal ions, other elements and salts in water can have a profound influence on the chemistry of the tea infusion.

The ancient texts also detail the use of urine as an “extraction” solvent, although this preparation method has not even been studied yet (Hsu, 2006). Based on the possible chemical processes occurring during the preparation step between *Artemisia annua* derived compounds and water ions/salts or urine metabolites, ions/salts, an inactive compound might become active or *vice versa*. By preparing a tea infusion at high temperatures, along with the presence of salts and microelements, a perfect environment for chemical reactions to take place is formed. For the traditionally used 24 h cold water or urine extraction the same reactions might take place although at a slower rate. During cold water extraction bacterial metabolisation may also occur affecting the chemical profile whereas this effect will probably not take place during preparation of the tea infusion due to high temperatures. Extraction with water (either cold or boiling) does have one advantage for scientific investigation, in that it simplifies the chemical profile considerably. It tends to extract only the more polar compounds (although the non-polar artemisinin is also extracted) (Van der Kooy and Verpoorte, 2011) and all compounds will be dissolved (no polymorphism possible). This might however explain why the C_{max} for artemisinin is reached after only 30 min if a tea infusion is consumed as compared to a couple of hours when pure artemisinin is consumed (Räth et al., 2004; Weathers et al., 2011).

2.3. Level 3: Influence of metabolism on the chemistry of the formulation

Prodrugs are compounds that tend to be inactive during *in vitro* tests, but become active only after *in vivo* metabolisation. To identify prodrugs in traditional medicines is very difficult if not impossible due to the sheer amount of chemical components present in the traditional medicine, of which the identity of most is unknown. Prodrugs can potentially play a big role in the overall efficacy of traditional medicines. The tea infusion of *Artemisia annua* contains many different compounds including the active principle artemisinin. The co-extracted compounds in the tea infusion may possibly have a synergistic effect on many different levels against *Plasmodium falciparum* for example: stimulating the immune system, inhibiting secondary infections (thereby improving the immune response reaction), affect the physical properties of the site of action (e.g. anticoagulants), affecting non-lethal biochemical processes in *Plasmodium falciparum* (e.g. efflux channel inhibitors), having a direct activity against *Plasmodium falciparum* but at a different target site, improving the bioavailability of the active compound etc. Some scholars have even suggested that traditional medicines work by restoring our unique microbiome and thereby enhance the body's ability to fend off non-related infections such as *Plasmodium falciparum*. All of these abovementioned factors together are called synergism. However, to prove synergism is very difficult, due to its inherent complexity and the fact that we predominantly use error prone *in vitro* bioassays to study synergism.

3. Chemistry of *Artemisia annua* infusions and the influence of the preparation method

Over 600 phytochemicals have been identified in *Artemisia annua* (Brown, 2010), all of which will be ingested when the leaves of *Artemisia annua* are consumed. There is currently no scientific literature available on the chemistry, effect of the preparation method (harvesting, drying, storage etc.) and overall

Fig. 2. Image from the Chinese *Materia Medica* depicting the investigation of plant material.

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bioavailability of any of these 600 compounds, excluding artemisinin, when *Artemisia annua* dried leaf material is administered. Weathers et al. (2011) and Elfawal et al. (2012) studied the bioavailability of artemisinin after administration of the whole leaf material in mice (this will be further discussed in Section 4). We can therefore only report on what is currently known on the water extracts prepared from *Artemisia annua*.

The *Artemisia annua* cold water extraction has been noted in the *Chinese Materia Medica* (Fig. 2) since antiquity (Carbonara et al., 2012). We assume that the tea infusion, which is currently being used, originated due to the preparation speed (minutes vs hours) even though this method was not originally described in the ancient texts. Both preparation methods use water without any reference to specific types of water e.g. rain water, river water etc., and according to the Chinese *Materia Medica* fresh *Artemisia annua* or *Artemisia apiacea* is soaked in either water or urine and then wrung out, leaving a juice which is then consumed (Hsu, 2006). No mention is made of the harvesting, drying and storage methods employed except that the fresh plant material was used. We can assume that *Artemisia* plant material could have been dried and stored in order to assure a year round supply, although we do not have any evidence to support this.

In the following sections we will report and discuss the available literature on the chemistry of the *Artemisia annua* infusions and decoctions and the influence that the preparation method can have on the chemistry of the final formulation.

3.1. Extraction efficiency and stability of artemisinin

Currently artemisinin is present at around 0.01–1.4% based on dry leaf mass in plants growing in the wild, although this might have been different in the past due to differing genetic and environmental factors. The main problem is that artemisinin has a low water solubility (~50 mg/L) at room temperature (Jansen, 2006; De Ridder et al., 2008). Heide (2006) proposed that this low water solubility is overcomed by the action of other constituents present in *Artemisia annua*. Räth et al. (2004) noted extraction efficiencies for artemisinin of greater than 70%, with boiling reducing the yield to around 30–53%. However, a previous study by Mueller et al. (2000) showed a decreased extraction efficiency of only 20–42%. The latter study only used the harvested leaves, whereas the former study used the entire plant. It is therefore possible that the compounds responsible for the enhanced aqueous solubility of artemisinin only occur in the stems and not in the leaves.

Wright et al. (2010) found that soaking the leaves and stems in water for 12 h followed by squeezing gave the most effective extraction yield of 72.6 mg/L, compared to 45.9 mg/L after 2 h and 14.5 mg/L for the dried herb infusion. They suggested that squeezing may cause the artemisinin concentration to increase, due to rupturing of the glandular trichomes containing most of the artemisinin. It may also extract essential oils from the trichomes that may aid in the solubility of artemisinin. The overall extraction efficiency of this study was however quite low (7.27% for 12 h, 4.51% for 2 h compared to 53.8% for the dried leaves infusion) (Wright et al., 2010). Silva et al. (2012) on the other hand observed high extraction efficiencies of 78–91% after boiling the dried leaves with no squeezing which are similar to those of Räth et al. (2004). Van der Kooy and Verpoorte (2011) studied the extraction efficiency of artemisinin at different temperatures and found as expected that with increasing temperatures the extraction efficiency of artemisinin increased. The highest extraction efficiencies (93%) were achieved when the water was kept at boiling point for 2–5 min. They did not find evidence to show other components increased the solubility of artemisinin, but rather that they may be decreasing the solubility (Van der Kooy and Verpoorte, 2011). Debnath et al. (2011) optimised a method with boiling water and obtained an extraction efficiency of around 84%, which is close to the results obtained by Van der Kooy and Verpoorte (2011). They also determined there to be a negligible difference between the extraction efficiency of distilled and tap water.

Van der Kooy and Verpoorte (2011) extended their study to encompass the stability of artemisinin in the tea infusion and found it to be stable over a 24 h period. Weathers and Towler (2012) confirmed this period of stability, noting that at room temperature artemisinin hardly degrades when present in the tea infusion. All of the abovementioned studies used deionised water, except for Debnath et al. (2011), and do not shed any light on the possible chemical reactions that can take place when "normal" water is used. Furthermore, no studies have been conducted on the possible chemical reactions that can take place or the extraction efficiency of urine, as this was the common extraction solvent in ancient times. This interaction between common water ions and salts, and also common metabolites present in urine, should be further investigated and its effect on the various biological activities ascribed to *Artemisia annua* studied.

3.2. Identification of other compounds in the Artemisia annua formulation

Many studies have focussed solely on the extraction and quantification of artemisinin without actually conducting a complete chemical profile of *Artemisia annua* formulations. Over the years, more than 600 secondary metabolites have been identified and reported for *Artemisia annua* (Brown, 2010) while only 37 compounds have been identified in tea infusions or cold water extracts, mainly consisting of caffeic acid derivatives, flavonoids, coumarins and artemisinin (Elford et al., 1987; Liu et al., 2006; D'angelo et al., 2009; De Oliveira et al., 2009; Weathers et al., 2011). The *Artemisia* genus as a whole has been reported to include a large variety of terpenes, phenols and acetylenes, along with coumarins and flavonoids (Carbonara et al., 2012). Liu et al. (2010) conducted a metabolomic investigation on *Artemisia afra* in relation to *Artemisia annua*, identifying 26 compounds, of which 23 were common in both species. Unfortunately, this study was conducted using organic solvents, when the actual medicinal formulation is prepared from water. During the same study they

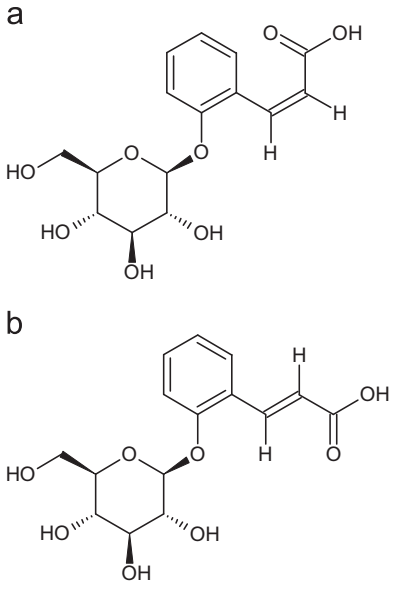

Fig. 3. (a) *cis*- and (b) *trans*-melilotoside.

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did however test the tea infusion for *in vitro* activity against *Plasmodium falciparum* (discussed in the next section).

Another study identified the active flavonoids casticin and artemetin to be present in the *Artemisia annua* tea infusion (Weathers and Towler, 2012). They did conclude that the low extraction efficiency of these flavonoids in the tea infusion ruled out synergism, and suggested using capsules containing the leaf material over the tea infusion (Weathers and Towler, 2012). Carbonara et al. (2012) also noted that flavonoids were not very abundant in the tea infusion, and observed the infusions to be rich in caffeic acid derivatives. They identified 25 phenolic compounds within *Artemisia annua* tea infusions and 27 phenolics in *Artemisia annua* water extracts after 1, 24 and 48 h periods. In our laboratory we recently identified two new compounds in the tea infusion, *cis*- and *trans*- melilotoside (Fig. 3), which is the first report of these compounds for any *Artemisia* spp. (Mouton et al., in press), and two known compounds scopoline and rutin. Table 1 lists the identities of all the compounds identified in *Artemisia annua* traditional formulations to date.

In order to better understand the biological activities ascribed to the use of *Artemisia annua*, a complete phytochemical investigation should be undertaken. This study should be conducted by preparing tea infusions with distilled and tap water from both fresh and dried *Artemisia annua* and *Artemisia apiacea.* Extraction with water and urine at room temperature should also be investigated. This should be followed by conducting a full degradation study and linking any degradation to enhanced or decreased biological activity.

4. Synergism and biological activity of the *Artemisia annua* formulation

4.1. Activity against Plasmodium spp. and synergism studies

In vitro studies performed on the *Artemisia annua* formulation include De Donno et al. (2012) who tested the *Artemisia annua* tea infusion against chloroquine-sensitive (D10) and resistant (W2) strains of *Plasmodium falciparum*, resulting in IC_{50} values of 1.11 ± 0.21 μg/mL and 0.88 ± 0.35 μg/mL against the D10 and W2 strains respectively. Based on these results it was concluded to be an effective treatment for *Plasmodium falciparum* infections and that the tea infusion exhibits some level of synergism improving its activity above that of pure artemisinin. These results were

Table 1
Compounds identified within the *Artemisia annua* traditional formulations.

nr	Chemical name	Preparation method	Place/Year of harvest	Reference
1	3-Caffeoylquinic acid	500 mg dried leaves, 13 mL boiling water, left to cool before filtering.	Italy 2008	Carbonara et al. (2012)
2	4-Caffeoylquinic acid			
3	3,4-Dicaffeoylquinic acid			
4	3,5-Dicaffeoylquinic acid			
5	4,5-Dicaffeoylquinic acid			
6	3-Feruloylquinic acid			
7	4-Feruloylquinic acid			
8	5-Feruloylquinic acid			
9	3,4-Diferuloylquinic acid			
10	3,5-Diferuloylquinic acid			
11	4,5-Diferuloylquinic acid			
12	3,5-Caffeoylferuloylquinic acid			
13	4-Caffeoyl-3,5-disuccinoylquinic acid			
14	6-C-arabinosyl-8-C-glucosyl apigenin			
15	6-C-glucosyl-8-C-arabinosyl apigenin			
16	Caffeic acid			
17	Chrysoeriolrutinoside			
18	Cirsilineol			
19	Isovitexin (6C-glucosyl apigenin)			
20	Jaceidin			
21	Luteolin-7-o-glucoside			
22	Patuletinglycoside			
23	Quinic acid			
24	Vitexin (8-C-glucosyl apigenin)			
25	Isoquercitrin	20 g dried leaves, 1 L boiling distilled water, allowed to cool for 15 min before filtering.	Brazil and Luxembourg 2012	Magalhaes et al. (2012)
26	Scopoletin			
27	Rosmarinic acid			
28	Artemisinin	9 g/L dried leaves kept at boiling point for 5-10 min and filtered.	China, Brazil and USA 2012.	Weathers and Towler (2012)
29	Artemetin			
30	Casticin			
31	Chrysosplenetin	5 g/L dried leaves in distilled boiling water, left for 15 min and filtered.	Amazonian ecosystem 2008	Silva et al. (2012)
32	Chrysosplenol D	5 g/L dried leaves in boiling water, left to cool for 15 min and filtered.	Italy 2008	De Donno et al. (2012)
33	Chlorogenic acid (5-caffeoylquinic acid)	See above respective references to preparation method and place/year of harvest.		Carbonara et al. (2012) and Magalhães et al. (2012)
34	*cis*-Melilotoside	90 mg dried leaves, 10 mL boiling water, boiled for 3 min and immediately filtered.	Germany 2010	Mouton et al., in press
35	*trans*-Melilotoside			
36	Scopoline			
37	Rutin			

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Table 2
Reported *in vitro* antimalarial activities of *Artemisia annua* tea infusions.

Artemisia annua formulation	Place/Year of Harvest	Plasmodium strain	Artemisinin content (mg/L)	IC50 of tea infusion (μg/mL)	IC_{50} of artemisinin (ng/mL)	Reference
4 differently prepared pounded and wrung juices	UK 2010	*Plasmodium falciparum* (NF54)	14.5–293.0	nd	0.21–0.59 (control* = 3.8)	Wright et al. (2010)
Tea infusion	Italy 2008	*Plasmodium falciparum* (D10)	Unclear	1.11	1.6–1.9 (control* = 6.1–7.1)	De Donno et al. (2012)
Tea infusion	Italy 2008	*Plasmodium falciparum* (W2)	Unclear	0.88	1.6–1.9 (control* = 6.1–7.1)	De Donno et al. (2012)
Tea infusion	South Africa 2006	*Plasmodium falciparum* (3D7)	ND	0.26	ND	Liu et al. (2010)
Tea infusions from 4 cultivars	Brazilia 2008	*Plasmodium falciparum* (field isolates)	40–46	nd	4.7–5.6 (control = unclear)	Silva et al. (2012)
Tea infusions of 16 different samples	Various	*Plasmodium falciparum* (3D7)	8.4–117.2	0.25–2.69	2.5–6.2 (control* = 5.5)	Mouton et al. (2013)

nd = not determined.

* Artemisinin used as a control.

consistent with those obtained by Räth et al. (2004) and Mueller et al. (2004). Artemisinin levels in the *Artemisia annua* tea (0.18 ± 0.02%) were deemed too low to be the sole cause of activity and it was proposed that other constituents increased the antimalarial activity, through possible synergism (De Donno et al., 2012).

Elford et al. (1987) suggested that the synergistic properties may come from flavonoids, which is possible given the activity of the tea was 3-fold better than that of pure artemisinin. Wright et al. (2010) conducted *in vitro* experiments with pounded and wrung juice of the fresh *Artemisia annua* herb and found the *in vitro* activity of artemisinin *in planta* to be 6–18 fold better than pure artemisinin alone (IC_{50} of artemisinin *in planta* 0.21–0.59 ng/mL vs artemisinin control = 3.8 ng/mL). The results of these experiments were confirmed with *in vivo* tests performed in mice where it was found that a dose of 18 mg/kg of artemisinin *in planta* suppressed parasitaemia by 95% while a 30 mg/kg dose of pure artemisinin suppressed the paraesatemia by 88%.

Silva et al. (2012) tested the effectiveness of the tea infusion against field isolated *Plasmodium falciparum* which were resistant to chloroquine. The infusions were determined to be an effective low toxicity treatment against chloroquine-resistant *Plasmodium falciparum*, having an IC_{50} of 0.11–0.14 μg/mL, however no synergistic effects were noted and thereby contradicting the results reported by De Donno et al. (2012) and Wright et al. (2010). Recently, Mouton et al. (2013) performed a similar *in vitro* study comparing the activity of pure artemisinin to 18 different *Artemisia annua* tea infusion samples and found good activity, but no synergistic effect for both the tea infusions and chloroform extracts. Table 2 lists the reported *in vitro* antimalarial activities of *Artemisia annua* tea infusions.

All of the above-mentioned *in vitro* studies made use of different *Artemisia annua* plant samples, different preparation methods and different *in vitro* bioassays. In order to perform a thorough *in vitro* synergism study on the *Artemisia annua* tea infusion, it would be an absolute necessity to first perform a full chemical analysis on the formulation. The highest level of synergism was reported by Wright et al. (2010) and therefore their preparation method should be used as a starting point (soaking *Artemisia annua* in water for 2 or 12 h at room temperature). This should be followed by comparing the chemical profile of the resulting sample with the chemical profile of a sample produced according to Mouton et al. (in press), where no synergism was reported (e.g. preparing a tea infusion). The chemical differences between these two sample sets should indicate which components are responsible for *in vitro* synergism. It will however be important to use the same plant material and to make use of the same *in vitro* bioassay.

In vivo studies performed with *Artemisia annua* tea infusions determined there to be inadequate amounts of artemisinin when compared to the WHO approved artemisinin dosage, in relation to the inhibition of *Plasmodium chabaudi chabaudi* (Atemnkeng et al., 2009). The same study also contradicts previous studies by stating that the tea did not decrease parasitaemia fast enough, though this could be a result of a different species of the *Plasmodium* parasite being used. An interesting study conducted by Chougouo et al. (2009) determined *Artemisia annua* infusions to have higher efficacy compared to the artesunate and artesunate + amodiaquine ACT treatments, necessitating the need to perform large scale *in vivo* trials given the contradictions in these published studies.

Studies conducted with purified compounds includes Magalhaes et al. (2012) who identified the main phenolic compounds in *Artemisia annua* to be chlorogenic and rosmarinic acids. Inhibition of the biotransformation intestinal and hepatic cytochrome P450 (CYP) enzymes, CYP3A4 and CYP1A1, was noted with rosmarinic acid decreasing the secretion of Interleukin-8 (IL-8) and IL-6 inflammatory cytokines when *Artemisia annua* extracts were assayed onto Caco-2 cells. These effects were determined to be free of toxicity and unique to the phenolic compounds within *Artemisia annua* tea infusions, which could synergise its antimalarial effects (Magalhaes et al., 2012). Reports of synergism of artemisinin with other metabolites were observed as a possibility by Carbonara et al. (2012) with about 40 flavonoids isolated from *Artemisia annua* leading to potentiation of artemisinin bioactivity. Weathers and Towler (2012) investigated the possibility of synergism by combining six methoxylated flavones individually with artemisinin and showed that the IC_{50} against *Plasmodium falciparum* was improved by 20–50%, indicating synergism. Ogwang et al. (2011) also noted the importance of flavonoids, given that *Artemisia annua* has a low content of artemisinin but is rich in flavonoid compounds. This observation shows the importance of flavonoids for the effectiveness of the tea infusion, however, it has also been shown that the tea infusion contains relatively low levels of flavonoids (Weathers and Towler, 2012).

4.2. The in vitro activity of Artemisia annua against other diseases

Artemisia annua also exhibits other less researched therapeutic properties. It is currently being prescribed by various NGOs such as ANAMED and IFBV-BELHERB for the treatment of malaria, cancer, AIDS, haemorrhoids and bronchitis, as well as skin and digestion problems (Anamed, 2012). A survey conducted by

Willcox et al. (2011) reported that around half of the respondents were also using *Artemisia annua* for the treatment of HIV/AIDS. Lubbe et al. (2012) investigated this, showing *Artemisia annua* tea infusions to be highly active against HIV (IC_{50}=2.0 μg/mL) with low toxicity even at high concentrations. In the same study pure artemisinin was found to be inactive at 25 μg/mL whereas an *Artemisia afra* infusion, known not to contain artemisinin, was also found to be highly active against HIV. According to Wu et al. (2001) HIV inhibition can be linked to the coumarin aesculetin, as well as the flavonoids arcapillin and isorhamnetin during studies performed on *Artemisia capillaris*. Further research is however needed to confirm the results reported by Lubbe et al. (2012) and to identify the compounds responsible for the observed anti-HIV activity. This should be followed by determining the variability of these compounds in different *Artemisia annua* cultivars and to study their pharmacokinetic properties during *in vivo* clinical studies. The *in vitro* anti-HIV activity of *Artemisia annua* tea infusions is discussed in detail by Van der Kooy and Sullivan (in press).

De Oliveira et al. (2009) and Costa et al. (2009) have tested *Artemisia annua* for activity against the *Toxoplasma gondii* parasite. In the study of De Oliveira et al. (2009), human foreskin fibroblasts and mice were infected with *Toxoplasma gondii* and treated with an *Artemisia annua* infusion, which effectively controlled the infection by directly inhibiting the parasite. These results were consistent with studies conducted by Jones-Brando et al. (2006) and D'angelo et al. (2009) which showed *Artemisia annua* to inhibit *Toxoplasma* replication. None of the other therapeutic uses of the *Artemisia annua* infusion has been studied in any detail.

There are a growing interest into the anticancer activity of artemisinin and its derivatives (Lai et al., 2013). There are however a number of problems associated with their anticancer properties such as a lower potency compared to existing chemotherapeutic agents and their short plasma half-lives. Therefore, to be effective a high dosage and frequent administration would be required (Lai et al., 2013). The search for selective anticancer agents has not been fruitful to date, mainly due to their toxicity or non-selectivity. With *Artemisia annua* and artemisinin we have a potential selective anticancer agent that has been used by millions of people (for the treatment of malaria) over a very long period of time without any major reports of toxicity. This fact alone should warrant further investigation into *Artemisia annua* and artemisinin in order to determine if it can be used as future anticancer drugs. There are however no scientific literature available on the anticancer activity of the traditional formulation of *Artemisia annua*. Some reports indicated that the bioavailability of artemisinin is increased when administered *in planta* (Weathers et al., 2011). This increased bioavailability (discussed in Section 5.2) could possibly aid in improving the anticancer properties of artemisinin when the traditional formulation is administered as compared to the single compound artemisinin. This aspect should be further investigated.

5. Pharmacological studies

5.1. Cure rate and recrudescence

The effectiveness of *Artemisia annua* for the treatment of malaria and other ailments has been long debated. The tea infusion has been called “misleading” (Jansen, 2006) due to the low artemisinin content within the plant. It has also been shown to lead to high recrudescence rates of *Plasmodium falciparum*, which could lead to the possible development of artemisinin resistant strains of *Plasmodium falciparum* (Mueller et al., 2004; Jansen, 2006). Previous *in vivo* studies conducted on *Plasmodium falciparum* have shown a rapid decrease in the number of parasites to undetectable limits, but also showed a high recrudescence rate (Mueller et al., 2004; Heide, 2006). Unfortunately these studies did not test the possibility that reinfection might be responsible for the observed high recrudescence rate. Mueller et al. (2004) was quite critical of the use of the *Artemisia annua* tea infusions in general, with their criticism forming the basis of many of the WHO's recommendations. However, their original study showed that parasites were cleared rapidly from the blood of 92% of the patients, but no follow up was carried out to determine the level of recrudescence and therefore the cure rate for this study is unknown (Mueller et al., 2000). Unfortunately, all of the published clinical studies associated with testing the *Artemisia annua* tea infusion have been conducted on a very small number of adults (De Ridder et al., 2008; Willcox et al., 2011), who could have been semi-immune to the parasite (Wright et al., 2010). Therefore care should be taken to link these results to the highest risk groups.

Contrary to this, Chougouo et al. (2009) showed the *Artemisia annua* treatment to have a 0% recrudescence rate compared to 12.5% and 14.3% for the ACTs artesunate and artesunate+amodiaquine respectively, which contradicts the conclusions of Mueller et al. (2004) and Heide (2006). Chougouo et al. (2009) confirmed the earlier work performed by ICIPE (2005), who reported a low recrudescence rate of around 10% for capsules containing dried *Artemisia annua*. However, they also suggested a treatment plan of 5–6 days followed by a secondary dosage another 5–6 days later to eliminate slow maturing parasites within the bloodstream. A similar strategy using the tea infusions rather than the dried plant material capsules was proposed by Ogwang et al. (2012), where it was shown that the prophylactic ingestion of *Artemisia annua* tea infusions once per week lowered the risk of contracting malaria by 55%. Ogwang et al. (2011) performed a similar study in which a Ugandan community ingested *Artemisia annua* tea once per week prophylactically to reduce the occurrence of malaria and fevers, resulting in 80% less clinical identifications of malaria.

The NGO company ANAMED claimed to have treated around 33,000 patients with *Artemisia annua* tea infusions with an overall cure rate of >93%. Unfortunately only a very small part of this study has been published (Willcox et al., 2011) and therefore data regarding diagnosis, subject profiles and how the cure rate was defined is not available. However, this unsubstantiated claim does provide us with an estimate of the scale of use and also the overall claimed effectiveness of the treatment. Table 3 lists all the results of pharmacological studies that has been performed and includes field reports from ANAMED.

Recrudescence and cure rate form the heart of the heated debate. This is mainly due to the limited available information which is apart from being very limited also very contradictory. The only way to settle this debate will therefore be to conduct a large scale *in vivo* trial, preferably designed and backed by the WHO.

5.2. Bioavailability of artemisinin

A study performed by Alin and Bjorkman (1994) reported that only 9 ng/mL of artemisinin was needed to inhibit *Plasmodium falciparum* growth *in vitro*. Jansen (2006) found that the concentrations of artemisinin within the *Artemisia annua* tea infusion were too low to achieve this, whereas Räth et al. (2004) observed artemisinin, administered in the form of a tea infusion, being absorbed fast and noted a C_{max} after 30 min of 240 ng/mL. The C_{max} of artemisinin was therefore found to be 26 times higher than the minimum concentration needed to inhibit the growth of *Plasmodium falciparum in vitro*. Unfortunately Räth et al. (2004) did not include a control group receiving pure artemisinin alone, but instead refers to the data of other studies performed using only pure artemisinin treatments. The main conclusion of this

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Table 3
In vivo analysis that has been performed with the *Artemisia annua* formulation.

Artemisia annua formulation used	*In vivo* test data	Health status	Treatment regime	Artemisinin dose	% Parasitaemia	C_{max} of artemisinin (ng/mL)	Recrudescence/ re-infection	Reference
Tea infusion	22 adult subjects	Malaria infected	1 L/day for 4 days	Unclear	100% free (day 4)	nd	0%	Mueller et al. (2000)
Tea infusion	48 adult subjects	Malaria infected	1 L/day for 4 days	Unclear	92% free (day 4)	nd	8%	Mueller et al. (2000)
Tea infusion	14 adult subjects	Healthy	1 L – single dose	94.5 mg	nd	240	nd	Räth et al. (2004)
Tea infusion	39 adult subjects	Malaria infected	1 L/day for 7 days	47.0 mg/day	77/34% free (day 7/35)	nd	23/66% (day 7/35)	Mueller et al. (2004)
Tea infusion	33 adult subjects	Malaria infected	1 L/day for 7 days	94.0 mg/day	70/30% free (day 7/35)	nd	30/70% (day 7/35)	Mueller et al. (2004)
Tea infusions	Various studies $n=33{,}000$ subjects	Malaria infected	Various	Various	$>95\%$ free	nd	$<5\%$	Anamed (2012)
Pounded juice	Mice ($n=$unknown)	*Plasmodium berghei* infected	500 μL once	9.0 mg/kg	Suppression of parasitaemia: 52%	nd	na	Wright et al. (2010)
Pounded juice	Mice ($n=$unknown)	*Plasmodium berghei* infected	500 μL twice	18.0 mg/kg	Suppression of parasitaemia: 95%	nd	na	Wright et al. (2010)
Pounded juice	Mice ($n=$unknown)	*Plasmodium berghei* infected	500 μL thrice	27.0 mg/kg	Suppression of parasitaemia: 96%	nd	na	Wright et al. (2010)
Dried leaves	Mice ($n=$unknown)	Healthy	Pelletized, single dose	30.7 μg	na	87	na	Weathers et al. (2011)
Tea infusion	Mice ($n=6$)	*Plasmodium chabaudi* infected	666 μL/day	22.0 μg/day	Level of parasiteamia 72%	na	na	Liu et al. (2010)
Dried leaves	Mice ($n=10$)	*Plasmodium chabaudi* infected	40 mg leafs, single dose	24.0 mg/kg	38% above 3% parasitaemia threshold (day 3)	nd	na	Elfawal et al. (2012)
Dried leaves	Mice ($n=6$)	*Plasmodium chabaudi* infected	200 mg leafs, single dose	120.0 mg/kg	0% above a 3% parasitaemia threshold (day 3)	nd	na	Elfawal et al. (2012)

nd=not determined, *na*= not applicable.

study was that although the C_{max} of artemisinin administered *in planta* was reached far quicker and was far higher than expected, the overall Area Under the Curve (AUC) of artemisinin administered *in planta* and pure artemisinin alone (data obtained from a different study) was similar.

Weathers et al. (2011) found similar results to Räth et al. (2004) after the administration of *Artemisia annua* leaves containing 31 μg of artemisinin *in planta*, leading to a C_{max} being reached after only 30 min. The administration form of the powderised leaves in this study by Weathers et al. (2011) raises the interesting possibility that a double C_{max} might be achieved. The powderised leaf material was mixed with water to form a slurry, which will dissolve artemisinin to some extent depending on the volume of water used, the contact time and the temperature (aqueous solubility of orthorhombic polymorph=20 mg/L vs triclinic=50 mg/L). The first C_{max} might be caused by the already dissolved artemisinin (after 30 min – similar to when the tea infusion is administered) while the second C_{max} might be caused by the remaining undissolved artemisinin *in planta* (after 2–3 h). *In vivo* studies performed in our own laboratory indicated that when people are given the leaf material to consume, a C_{max} is reached after 2–3 h (unpublished results). Unfortunately Weathers et al. (2011) only determined the artemisinin plasma concentration up to 1 h after administration. We can therefore state that it is quite possible that the C_{max} reported by Weathers et al. (2011) is due to the already dissolved artemisinin in the tablets/slurry and that we can expect another C_{max} to be reached after 2–3 h.

Weathers et al. (2011) also showed that 31 μg of pure artemisinin was not detectable in the circulatory system of mice after 1 h, while 1400 μg was detectable after 1 h at levels of ≥ 74 ng/mL. Compared to this the administration of 31 μg of artemisinin *in planta* was detectable at a level of 84 ng/mL after 1 h. They therefore concluded that the bioavailability of artemisinin administered in the form of powderised plant material (although water was used during the production process) is far greater (~45 fold) compared to pure artemisinin. Elfawal et al. (2012) furthered this study by comparing the *in vivo* activity of a slurry containing different quantities of powderised *Artemisia annua* leaf material and pure artemisinin. They determined the slurry (*Artemisia annua* leaves and water) to be far more effective against the parasites than pure artemisinin. This study again shows that the mixture of leaf material and water might lead to a double C_{max} being achieved which might be an effective way to clear the *Plasmodium falciparum* parasites.

5.3. Artemisinin metabolism

A recent study stated that artemisinin has a low oral bioavailability in humans (Magalhaes et al., 2012), which is consistent with previous reports (Mueller et al., 2000; Räth et al., 2004). However, Magalhaes et al. (2012) also suggested the possibility that the inhibition of CYP enzymes by flavonoids and other constituents may increase the bioavailability of artemisinin. This has been noted in other studies where flavonoids possibly play a role in increasing the bioavailability of artemisinin (Räth et al., 2004). It is important to note that the inhibition of CYPs 2B6 and 3A4 could lead to *in vivo* increases in the bioavailability of artemisinin (Magalhaes et al., 2012). Weathers et al. (2011) also observed that ingesting powderised whole leaf material could increase artemisinins effectiveness, as well as decreasing the chance of resistance from developing. This is contrary to many publications concluding that *Artemisia annua*

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treatment is an ineffective mono-therapy, and might cause resistance (Mueller et al., 2000, 2004; Räth et al., 2004). Both ICIPE (2005) and Chougouo et al. (2009) have shown the recrudescence rates for the dried plant material capsules at a maximum of 10%. A recent paper published by Elfawal et al. (2012) administering *Artemisia annua* plant material to rats, determined it to be more effective than the purified artemisinin.

The metabolism of artemisinin by liver microsomes through CYP 2B6, with secondary contribution of CYP3A4 (De Ridder et al., 2008; Magalhaes et al., 2012) is important when trying to understand the bioavailability of artemisinin and other *Artemisia annua* constituents. Artemisinin derivatives, artesunate, artemether and arteether, are all metabolised into dihydroartemisinin (DHA) (Balint, 2001) which is just as potent as its parent compounds, but has a longer half-life of approximately 4–11 h. Artemisinin on the other hand is not metabolised into DHA but rather into inactive metabolites deoxyartemisinin and dihydroxydeoxyartemisinin (Balint, 2001). Artemisinin itself suffers from having a short half-life and it is known not to be metabolised into DHA (Lee and Hufford, 1990).

It would however be a major improvement if artemisinin could be derivatised into DHA before being consumed in the form of a tea or as whole plant material. This derivatisation of artemisinin into DHA might not be too far-fetched as Sibmooh et al. (2001) showed that artemisinin degrades into DHA in aqueous solutions in the presence of metal ions through metal catalysed reduction. This reaction can theoretically also take place when the *Artemisia annua* tea infusion is prepared by using normal tap water (containing metal ions) instead of deionised water. This was confirmed by Carbonara et al. (2012) who identified DHA in a tea infusion. Our understanding of this mechanism is important as longer lasting drugs are necessary to prevent resistance and ensure elimination of parasites. This relatively simple chemical reaction could occur under field conditions but not under laboratory conditions and therefore might explain the claimed enhanced activity of the tea infusion. This potentially extremely important aspect has not been fully studied yet.

6. Conclusions and future perspectives

Our main objective with this review was to collate all information available on the traditional *Artemisia annua* formulation and to highlight the complexity and the interplay between the chemistry of the treatment (medicinal plant), the chemical influence of the preparation method and the pharmacology and general "absorption, distribution, metabolism, excretion and toxicity" properties of the formulation in the patient. Based on this, very little scientific data currently exist on the traditional formulation consisting of *Artemisia annua* on each of these three levels. We have also shown that there are vast inconsistencies in the limited information presently available. The following number of aspects is important for future research into *Artemisia annua* and highlights the current gaps in the scientific literature. It is also important that these aspects are considered with respect to the ongoing debate between those advocating herbal antimalarial treatment and those recommending ACT's.

a. *The traditional preparation method involved soaking **fresh** Artemisia annua or Artemisia apiacea plant material in water or urine, wringing out the plant material and consuming the juice.*
   - This has not been studied in any detail before. Both species should undergo in-depth chemical and pharmacological analysis. For example: it was shown that the fresh *Artemisia annua* plant material contain pyrethrins (Singh and Sarin, 2011) which exhibits a high level of *in vitro* antiplasmodial activity (Hata et al., 2011). These compounds could not yet be identified in the dried leaves of *Artemisia annua* (however, these compounds might also originate from commercial pesticide use).

b. *Currently a tea infusion of dried Artemisia annua leaves is prepared and consumed and/or only the leaves of Artemisia annua are consumed as a whole.*
   - This deviates from the traditional use and has not yet been studied in any detail. The main debate here is the overall dose of artemisinin received, which is deemed too low, compared to the WHO recommended dose of artemisinin. This aspect is rather complicated but is simplified by the fact that only the dose of a single compound is looked at. This of course excludes the possibility of synergism and prodrugs playing any part in the overall efficacy. Contrary to popular belief, both parties in this debate also suffer from a similar problem. Indeed it will be extremely difficult to standardise the exact dose of artemisinin in the plant material (or tea infusion) but it also appears to be difficult to assure an exact dose of artemisinin and/or its main derivatives in the ACT regimen, due to thermal instability (Haynes et al., 2007) and due to the big, growing problem of counterfeit drugs. On top of this, the variability of patient response to a fixed dose of *Artemisia annua* formulations and ACTs is extremely high. The question needs to be asked: what is the use of assuring an exact dose of 500 mg of artemisinin if the bioavailability in patient one will be <25 ng/mL and in patient two 250 ng/mL? This issue needs to be addressed by both parties in this debate.

c. *Artemisinin dissolves well in water at high temperatures ($>90\%$ extraction efficiency).*
   - There is a perception that artemisinin does not dissolve in water. This perception is wrong. Artemisinin dissolves, depending on the polymorphic type, at between 20 and 50 mg/L at room temperature and at more than 200 mg/L in boiling water. Furthermore, artemisinin appears to become supersaturated at high temperatures and remain dissolved at much higher concentrations than expected even when the temperature of the solution returns to room temperature (Van der Kooy and Verpoorte, 2011).

d. *When the whole plant material is consumed all available artemisinin will be ingested.*
   - This aspect negates the debate around the extraction efficiency of water. However, the few studies performed on this aspect administered a slurry consisting of leaves and water. It is therefore theoretically possible that the preparation method might have extracted artemisinin at 20–50 mg/L into the water causing the observed C_{max} of artemisinin after 30 min. The remaining undissolved artemisinin in the plant material may possibly cause a second C_{max} at between 2 and 4 h – this should be further investigated.

e. *In the plant itself over 600 metabolites have been identified, all of which might play a role in potential synergism or might be activated upon in vivo metabolisation (prodrugs).*
   - The reported literature is contradictory as evident from Table 2. This aspect calls for a more holistic approach. In order to study this and to identify the synergistic compounds or prodrugs an *in vivo* study should be performed coupled to untargeted metabolomic analysis (Van der Kooy et al., 2009). This should give us insight into which compounds, and in what form, are present in the circulatory system at various time points.

f. *In the tea infusion and cold water extract only 37 compounds have been identified of which two compounds, cis- and trans-melilotoside, are new for the species. The melilotosides are known to be active against diarrhoea causing pathogens and provide further importance to study this multifunctional medicinal plant.*
   - The *Artemisia annua* tea infusion is currently being used for a multitude of ailments. An in-depth chemical analysis in order

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to identify and quantify the secondary metabolites is therefore urgently needed. This in-depth analysis should not only make use of MS technologies (as was done before) but also NMR analysis in order to unravel stereochemistry and also identify new compounds. A study performed in our laboratories (Mouton et al. in press) identified *cis*- and *trans*-melilotoside which has been shown to be active against the diarrhoea causing pathogens *Entamoeba histolytica* and *Giardia lamblia* (IC_{50} = 12.5 and 16.8 μg/mL respectively) indicating the traditional use of *Artemisia annua* for the treatment of diarrhoea might be effective (Calzada et al., 2003).

g. *Ions, salts and metabolites (e.g. urea) present in water and urine might play a role in artemisinin extraction or derivatisation into a more active compound (e.g. DHA). This process of chemical derivatisation can also happen to any other compound in the infusion or decoction.*
   - The importance of this is that if any compound is derivatised into a more active/stable/pharmacological superior compound, it could explain the continued use of *Artemisia annua* over a 2000 year period, but also why no *Plasmodium falciparum* resistance has emerged in the past. This aspect has a twofold implication: if correct, it will explain how this traditional medicine work and vindicate the traditional use of *Artemisia annua,* and it might also lead to a pharmacological superior lead compound that could be further developed. This has the potential to satisfy both parties in this debate and hopefully will bring these two scientific fields closer together.

h. *The tea infusion has been shown to be active against Plasmodium falciparum in vitro with some studies showing that synergism plays a role while other studies reporting that synergism do not play a role.*
   - Various studies have reported on the *in vitro* activity of the *Artemisia annua* tea infusion. Generally speaking the *in vitro* activity against *Plasmodium falciparum* is not debated. The main issue here is whether artemisinin is the only active compound or not. This aspect could not yet be clearly shown, and should be investigated in more detail (as explained in Section 4.1) during *in vivo* analysis as opposed to *in vitro* analysis alone. For the time being this aspect of the debate will continue.

i. *In vitro studies on the tea infusion provided evidence that it is highly active against HIV 1 and -2, and active against the parasite Toxoplasma gondii.*
   - The alternative uses of *Artemisia annua* are being overlooked. This plant has been used against various diseases and it was recently shown to contain potent anti-HIV compound(s). To put this into perspective: drug discovery is a time consuming and expensive exercise with a high failure rate (failure usually due to toxicity). Even with existing approved drugs this problem persists e.g. more than 200,000 people die annually in the European Union due to adverse drug reactions (Archibald and Coleman, 2012). With *Artemisia annua* we have a medicinal plant that is currently undergoing a large-scale **un**controlled *in vivo* clinical trial for the treatment of malaria and has already shown us that no or very little toxicity exists. Due to this, we can confidently assume that the anti-HIV compounds will probably have a low toxicity (but may also have low efficacy at the quantities present in the tea infusion) and their identification should therefore be a matter of urgency as described in Section 4.2.

j. *The C_{max} of artemisinin is enhanced by up to 19-fold if the tea infusion is consumed (in vivo in people) and up to 45-fold if the plant material is consumed (in vivo in mice).*
   - The pharmacological aspects of the traditional formulation are superior to the single compound artemisinin. The C_{max} of artemisinin is far higher than expected although the AUC appears to be similar based on the results reported in one study. This aspect should be studied in a large *in vivo* trial in order to understand the pharmacokinetic and dynamic properties of not only artemisinin but also of all other important phytochemicals in the *Artemisia annua* formulation. Here we also have to keep in mind the potential anticancer application of the traditional formulation.

k. *There are vast inconsistencies regarding the reported recrudescence rates and the question remains if the reported recrudescence is in fact recrudescence or reinfection.*
   - Even with the use of a multitude of different *Artemisia annua* plant samples, with a large degree of variation in artemisinin content, the tea infusion appears to be more effective than expected. There are some reports of a high recrudescence rates while others report a very low rate (Section 5.1). Unfortunately the small scale studies that were performed did not test for reinfection but rather assumed recrudescence. Only one large scale study has been conducted where a large number of people ($n = > 33{,}000$) have been treated with a reported cure rate of $> 93\%$. Although unsubstantiated and without the proper controls, this report if true indicates that the tea infusion might be equivalent or even better than current ACT treatments. The only way to settle this debate will be to perform large scale *in vivo* clinical trials.

l. *Artemisia annua and artemisinin are remarkably non-toxic and both are being used on a large scale globally.*
   - This is an important aspect. *Artemisia annua* is a perfect model medicinal plant with good *in vitro* activity and claimed *in vivo* activity against various diseases with an apparent lack of toxicity. It should therefore undergo in-depth scientific analysis in order to unravel how medicinal plants work, what the influence of synergism and prodrugs are on the overall efficacy and how to formulate a medicinal plant to become a mainstream inexpensive weapon in the fight against disease. If rigorous scientific investigation reveals that *Artemisia annua* can be used against (some of) these diseases the next step will be to investigate and optimise a specific formulation in order to assure an increased compliance by high risk groups e.g. young children.

The WHO is understandably reluctant to endorse the traditional use of *Artemisia annua* formulations, due to the limited available knowledge and the vast inconsistencies that are found in the scientific literature. In order to take these inconsistencies away and to be able to make informed decisions, future scientific research should include the following:

- A study into the chemical reactions that can take place between *Artemisia annua* compounds, focussing on artemisinin, and urine metabolites as well as common water ions and salts.
- A detailed and in-depth chemical analysis (preferably with nuclear magnetic resonance and mass spectrometry based metabolomic approaches (Van Der Kooy et al., 2009)) of the tea infusion and decoction using all different preparation methods (normal water and urine) and both *Artemisia annua* and *Artemisia apiacea* fresh and dried plant material.
- Chemical constituents identified in *Artemisia annua* and *Artemisia apiacea* should undergo a detailed activity/toxicity analysis against all the reported diseases (e.g. malaria, HIV, cancer).
- A large *in vivo* experiment should be performed to study the pharmacokinetics and dynamics of artemisinin administered in the various formulations as described in this review. Metabolomic approaches should also be applied in order to

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determine which other compounds play a role in possible synergism and if any prodrugs can be identified.
- A large *in vivo* clinical trial should be performed for the different diseases to study the effectiveness of an optimised formulation.

In summary, we know that a vast number of people are using *Artemisia annua* in various forms for the treatment of various diseases. They will also continue to do so due to the availability, low cost, apparent efficacy but also due to a (growing) lack of trust of western based medicines. We know that *Artemisia annua* has the potential to help solve some of the biggest health problems in the world. Due to a lack of (and gaps in) scientific data as presented in this review paper and the minimal investment to date into research into the traditional formulation, we do indeed run the risk of losing the artemisinins to resistant *Plasmodium falciparum* parasites-albeit from using tea infusions or ACTs (due to counterfeit ACTs and thermal instability) or a combination of both. We need to understand which components are needed for optimum efficacy, taking into account the influence of the preparation method and metabolisation processes, and the best way of administration to achieve the desired clinical end points. We are also aware that we are making high level claims for a traditional medicine which means we are entering the playground of the pharmaceutical industry. The main problem or limitations might therefore not only be scientific but also economic or political. Is the answer to the lack of interest in the traditional use of the *Artemisia annua* formulation that we should maybe first come up with a decent business plan?

Acknowledgements

We would like to thank Dr. Pierre Lutgen from IFBV-BELHERB and Dr. Martin Hirt from ANAMED for comments on this manuscript. Unfortunately no comments were received from the WHO African office. We also like to thank Miss. Ros Priest and Suzannah Bourchier for proof reading this manuscript and CompleMed, University of Western Sydney for providing the facilities to complete this work.

References

Alin, M.H., Bjorkman, A., 1994. Concentration and time dependency of artemisinin efficacy against *Plasmodium falciparum in vitro*. American Journal of Tropical Medicine and Hygiene 50, 771–776.

Anamed. Aktion Natürliche Medizin, 2012. Germany. ⟨http://www.anamed.net⟩ [accessed 16.12.12].

Archibald, K., Coleman, R., 2012. How Human Biology can Prevent Drug Deaths. ⟨http://www.newscientist.com/article/mg21628950.200-how-human-biology-can-prevent-drug-deaths.html⟩ [accessed 1.3.13].

Atemnkeng, M.A., Chimanuka, B., Dejaegher, B., Heyden, Y.V., Plaizier-Vercammen, J., 2009. Evaluation of *Artemisia annua* infusion efficacy for the treatment of malaria in *Plasmodium chabaudi chabaudi* infected mice. Experimental Parasitology 122, 344–348.

Balint, G.A., 2001. Artemisinin and its derivatives: an important new class of antimalarial agents. Pharmacology & Therapeutics 90, 261–265.

Brown, G.D., 2010. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). Molecules 15, 7603–7698.

Calzada, F., Velazquez, C., Cedillo-Rivera, R., Esquivel, B., 2003. Antiprotozoal activity of the constituents of *Teloxys graveolens*. Phytotherapy Research 17, 731–732.

Carbonara, T., Pascale, R., Argentieri, M.P., Papadia, P., Fanizzi, F.P., Villanova, L., Avato, P., 2012. Phytochemical analysis of a herbal tea from *Artemisia annua* L. Journal of Pharmaceutical and Biomedical Analysis 62, 79–86.

Chan, K.-L., Yuen, K.-H., Takayanagi, H., Janadasa, S., Peh, K.-K., 1997. Polymorphism of artemisinin from *Artemisia annua*. Phytochemistry 46, 1209–1214.

Chougouo, K.R.D., Kouamouo, J., Moyou, S.R., Penge, O.P., 2009. Comparative study of the quality and efficiency of artemisinin drug based and *Artemisia annua* grown in Cameroon. In: Proceedings of the MIM conference. Nairobi, Kenya. [Mim15225512].

Costa, I.N., Angeloni, M.B., Santana, L.A., Barbosa, B.F., Silva, M.C., Rodrigues, A.A., Rostkowsa, C., Magalhaes, P.M., Pena, J.D., Silva, D.A., Mineo, J.R., Ferro, E.A., 2009. Azithromycin inhibits vertical transmission of *Toxoplasma gondii* in *Calomys callosus* (Rodentia: Cricetidae). Placenta 30, 90–884.

D'angelo, J., Bordon, C., Posner, G.H., Yolken, R., Jones-Brando, L., 2009. Artemisinin derivatives inhibit *Toxoplasma gondii in vitro* at multiple steps in the lytic cycle. Journal of Antimicrobial Chemotherapy 63, 146–150.

De Donno, A., Grassi, T., Idolo, A., Guido, M., Papadia, P., Caccioppola, A., Villanova, L., Merendino, A., Bagordo, F., Fanizzi, F.P., 2012. First-time comparison of the *in vitro* antimalarial activity of *Artemisia annua* herbal tea and artemisinin. Transactions of the Royal Society of Tropical Medicine and Hygiene 106, 696–700.

De Oliveira, T.C., Silva, D.A., Rostkowska, C., Bela, S.R., Ferro, E.A., Magalhaes, P.M., Mineo, J.R., 2009. *Toxoplasma gondii*: effects of *Artemisia annua* L. on susceptibility to infection in experimental models *in vitro* and *in vivo*. Experimental Parasitology 122, 233–241.

De Ridder, S., Van Der Kooy, F., Verpoorte, R., 2008. *Artemisia annua* as a self-reliant treatment for malaria in developing countries. Journal of Ethnopharmacology 120, 302–314.

Debnath, C., Dobernig, A., Saha, P., Ortner, A., 2011. Electrochemical determination of artemisinin in *Artemisia annua* L. herbal tea preparation and optimization of tea making approach. Journal of the Korean Chemical Society 55, 57–62.

Elfawal, M.A., Towler, M.J., Reich, N.G., Golenbock, D., Weathers, P.J., Rich, S.M., 2012. Dried whole plant *Artemisia annua* as an antimalarial therapy. PLoS One 7, 1–7.

Elford, B.C., Roberts, M.F., Phillipson, J.D., Wilson, R.J., 1987. Potentiation of the antimalarial activity of Qinghaosu by methoxylated flavones. Transactions of the Royal Society of Tropical Medicine and Hygiene 81, 434–436.

Hata, Y., Zimmermann, S., Quitschau, M., Kaiser, M., Hamburger, M., Adams, M., 2011. Antiplasmodial and antitrypanosomal activity of pyrethrins and pyrethroids. Journal of Agriculture and Food Chemistry 59, 9172–9176.

Haynes, R.K., Chan, H.W., Lung, C.M., Ng, N.C., Wong, H.N., Shek, L.Y., Williams, I.D., Cartwright, A., Gomes, M.F., 2007. Artesunate and dihydroartemisinin (DHA): unusual decomposition products formed under mild conditions and comments on the fitness of DHA as an antimalarial drug. ChemMedChem 2, 1448–1463.

Heide, L., 2006. Artemisinin in traditional tea preparations of *Artemisia annua*. Transactions of the Royal Society of Tropical Medicine and Hygiene 100, 802.

Hsu, E., 2006. The history of Qing Hao in the Chinese Materia Medica. Transactions of the Royal Society of Tropical Medicine and Hygiene 100, 505–508.

Hsu, E., 2010. Qinghao (Herba *Artemisiae annuae*) in the Chinese materia medica. In: Hsu, E., Harris, S. (Eds.), Plants Health and Healing. Berghahn Books, pp. 83–114.

ICIPE: International Centre of Insect Physiology and Ecology, 2005. Whole-Leaf *Artemisia annua*-Based Antimalarial Drug: Report On Proof-Of-Concept Studies. Nairobi, Kenya.

Jansen, F.H., 2006. The herbal tea approach for artemisinin as a therapy for malaria? Transactions of the Royal Society of Tropical Medicine and Hygiene 100, 285–286.

Jefford, C.W., Burger, U., Millasson-Schmidt, P., Bernardinelli, G., Robinson, B.L., Peters, W., 2000. Epiartemisinin, a remarkably poor antimalarial: implications for the mode of action. Helvetica Chimica Acta 83, 1239–1246.

Jones-Brando, L., D'angelo, J., Posner, G.H., Yolken, R., 2006. *In vitro* inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. Antimicrobial Agents and Chemotherapy 50, 4206–4208.

Lai, H.C., Singh, N.P., Sasaki, T., 2013. Development of artemisinin compounds for cancer treatment. Investigational New Drugs. 31, 230–246.

Lee, I.-S., Hufford, C.D., 1990. Metabolism of antimalarial sesquiterpene lactones. Pharmacology and Therapeutics 48, 345–355.

Liu, C., Zhao, Y., Wang, Y., 2006. Artemisinin: current state and perspectives for biotechnological production of an antimalarial drug. Applied Microbiological Biotechnology 72, 11–20.

Liu, N.Q., Cao, M., Frederich, M., Choi, Y.H., Verpoorte, R., Van Der Kooy, F., 2010. Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. Journal of Ethnopharmacology 128, 230–235.

Lubbe, A., Seibert, I., Klimkait, T., Van Der Kooy, F., 2012. Ethnopharmacology in overdrive: the remarkable anti-HIV activity of *Artemisia annua*. Journal of Ethnopharmacology 141, 854–859.

Magalhães, P.M., Dupont, I., Hendrickx, A., Joly, A., Raas, T., Dessy, S., Sergent, T., Schneider, Y.J., 2012. Anti-inflammatory effect and modulation of Cytochrome P450 activities by *Artemisia annua* tea infusions in Human intestinal Caco-2 cells. Food Chemistry 134, 864–871.

Mouton, J., Jansen, O., Frédérich, M., Van der Kooy, F., 2013. Is artemisinin the only antiplasmodial compound in the *Artemisia annua* tea infusion? An *in vitro* study. Planta Medica 79, 468–470.

Mouton, J., Sullivan S.E., Van der Kooy, F. Identification of cis- and trans- melilotoside within an Artemisia annua tea infusion. European Journal of Medicinal Plants, in press.

Mueller, M.S., Karhagomba, I.B., Hirt, H.M., Wemakor, E., 2000. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. Journal of Ethnopharmacology 73, 487–493.

Mueller, M.S., Runyambo, N., Wagner, I., Borrmann, S., Dietz, K., Heide, L., 2004. Randomized controlled trial of a traditional preparation of *Artemisia annua* L. (annual Wormwood) in the treatment of malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene 98, 318–321.

Murray, C.J.L., Rosenfeld, L.C., Lim, S.S., Andrews, K.G., Foreman, K.J., Haring, D., Fullman, N., Naghavi, M., Lozano, R., Lopez, A.D., 2012. Global malaria mortality between 1980 and 2010: A systematic analysis. The Lancet 379, 413–431.

Please cite this article as: van der Kooy, F., Sullivan, S.E., The complexity of medicinal plants: The traditional *Artemisia annua* formulation, current status and future perspectives. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.08.021

Newton, P.N., Green, M.D., Mildenhall, D.C., Plancon, A., Nettey, H., Nyadong, L., Hostetler, D.M., Swamidoss, I., Harris, G.A., Powell, K., 2011. Poor quality vital anti-malarials in Africa – an urgent neglected public health priority. Malaria Journal 10, 352.

Ogwang, P.E., Ogwal, J.O., Kasasa, S., Ejobi, F., Kabasa, D., Obua, C., 2011. Use of *Artemisia annua* L. infusion for malaria prevention: mode of action and benefits in a Ugandan community. British Journal of Pharmaceutical Research 1, 124–132.

Ogwang, P.E., Ogwal, J.O., Kasasa, S., Olila, D., Ejobi, F., Kabasa, D., Obua, C., 2012. *Artemisia annua* L. infusion consumed once a week reduces risk of multiple episodes of malaria: a randomised trial in a Ugandan community. Tropical Journal of Pharmaceutical Research 13, 445–453.

Phyo, A.P., Nkhoma, S., Stepniewska, K., Ashley, E.A., Nair, S., Mcgready, R., Ler Moo, C., Al- Saai, S., Dondorp, A.M., Lwin, K.M., Singhasivanon, P., Day, N.P.J., White, N.J., Anderson, T.J.C., Nosten, F., 2012. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. The Lancet 379, 1960–1966.

Räth, K., Taxis, K., Walz, G., Gleiter, C.H., Li, S.-M., Heide, L., 2004. Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of *Artemisia annua* L. (annual Wormwood). American Journal of Tropical Medicine and Hygiene 70, 128–132.

Sibmooh, N., Udomsangpetch, R., Kujoa, A., Chantharaksri, U., Mankhetkorn, S., 2001. Redox reaction of artemisinin with ferrous and ferric ions in aqueous buffer. Chemical and Pharmaceutical Bulletin 49, 1541–1546.

Silva, L.F.R.E., De Magalhães, P.M., Costa, M.R.F., Alecrim, M.D.G.C., Chaves, F.C.M., Hidalgo, A.D.F., Pohlit, A.M., Vieira, P.P.R., 2012. *In vitro* susceptibility of *Plasmodium falciparum* Welch field isolates to infusions prepared from *Artemisia annua* L. cultivated in the Brazilian amazon. Memórias do Instituto Oswaldo Cruz 107, 859–866.

Singh, A., Sarin, R., 2011. Pyrethins from *in vivo* and *in vitro* cultures of *Artemisia annua*. Indian Journal of Environmental Sciences 15, 35–37.

Van der Kooy, F., Maltese, F., Choi, Y.H., Kim, H.K., Verpoorte, R., 2009. Quality control of herbal material and phytopharmaceuticals with MS and NMR based metabolic fingerprinting. Planta Medica 75, 763–775.

Van der Kooy, F., Verpoorte, R., 2011. The content of artemisinin in the *Artemisia annua* tea infusion. Planta Medica 77, 1754–1756.

Van der Kooy F., Sullivan S.E. Reverse pharmacology and drug discovery: Artemisia annua and its anti-HIV activity, In: Aftab T., Ferreira J., Khan M.M.A. and Naeem M., (Eds.), Artemisia annua – Pharmacology and Biotechnology, Springer, (in press).

Weathers, P.J., Arsenault, P.R., Covello, P.S., McMickle, A., Teoh, K.H., Reed, D.W., 2011. Artemisinin production in *Artemisia annua*: studies *in planta* and results of a novel delivery method for treating malaria and other neglected diseases. Phytochemistry Reviews 10, 173–183.

Weathers, P.J., Towler, M.J., 2012. The flavonoids casticin and artemetin are poorly extracted and are unstable in an *Artemisia annua* tea infusion. Planta Medica 78, 1024–1026.

World Health Organisation, 2012. WHO Position Statement on Effectiveness of Non-Pharmaceutical Forms of *Artemisia annua* L. Against Malaria. ⟨http://www.who.int/malaria/position_statement_herbal_remedy_artemisia_annua_l.pdf⟩ (accessed 28.2.13).

Willcox, M.L., Burton, S., Oyweka, R., Namyalo, R., Challand, S., Lindsey, K., 2011. Evaluation and pharmacovigilance of projects promoting cultivation and local use of *Artemisia annua* for malaria. Malaria Journal 10, 84.

Wright, C.W., Linley, P.A., Brun, R., Wittlin, S., Hsu, E., 2010. Ancient Chinese methods are remarkably effective for the preparation of artemisinin-rich extracts of Qing Hao with potent antimalarial activity. Molecules 15, 804–812.

Wu, T.-S., Tsang, Z.-J., Wu, P.-L., Lin, F.-W., Li, C.-Y., Teng, C.-M., Lee, K.-H., 2001. New constituents and antiplatelet aggregation and anti-HIV principles of *Artemisia capillaries*. Bioorganic & Medicinal Chemistry 9, 77–83.

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