Medicinal Plants for Malaria: A realistic use of herbals?

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Malaria, in addition to being the most pernicious parasitic disease of humans, is also the most prevalent. Current statistics suggest that malaria kills between 2.7-3 million people each year, with the majority being children under the age of 5. [1] *Plasmodium* spp. has generated resistance to all classes of antimalarial drugs and as a result there has been a doubling of malaria-attributable child mortality in eastern and southern Africa.[2] Disturbingly, malaria is so common in certain tropical areas that "low transmission areas" are defined as a person acquiring *Plasmodium* spp. infection less than 3 times a year. Conversely, in some tropical areas new malaria infections are acquired more than once each day and can be asymptomatic.[3] Previous estimates suggest that it requires less than 10 *Plamodium* sporozoite parasites injected by an infected mosquito in order to establish malaria infection.[4] Current statistics suggest that approximately 300 million people on the planet are infected with *Plasmodium* spp.

History of malaria

The discovery of the parasite itself is credited to the military surgeon Charles Louis Alphonse Laveran in 1880. While stationed in Algeria, he observed the pigment in cyst-like bodies within red blood cells, however, it took him some time to realize that these bodies were the parasite.[5] Of the 4 species of malaria parasites that infect humans -- *Plasmodium falciparum, P. vivax, P ovale , P.malariae* -- the most deadly is *P. falciparum.* If falciparum malaria is treated appropriately, the mortality is a mere 0.1%.[3] However, *P. falciparum* parasites, especially from Southeast Asia, are particularly known for developing drug resistant strains and these strains can produce a mortality rate of 15-20%.[6] Unfortunately, this statistic is often disregarded as a problem for developing countries.

Malaria was once known as *ague,* a term of Italian origin (from the Latin *acuta* meaning sharp, as in an acute fever). Although primarily associated with tropical climates, malaria was historically also present in non-tropical climates, from Britain to the southeastern United States.* In the southeastern United States, malaria was a scourge, prompting the development of the Center for Disease Control and Prevention (CDC) in Atlanta to investigate the prevention of malaria during WWII [7]. Malaria's symptoms are so distinct that historians have traced its presence to ancient civilizations dating from 1000 BCE. The symptoms—paroxysmal fever, shaking chills, sweating—have been described in the Hippocratic Collection.[5] So incapacitating is the disease that the expansion of civilizations and empires in the past depended on a cure for the debilitating fevers of malaria. It is speculated that Alexander the Great—whose armies conquered much of what was then the civilized world—may have died of malaria in Babylonia.[8]

As the British Empire expanded into tropical regions of Africa, India and the Caribbean, so did the risk of exposure to malaria. By far one of the most common, debilitating and often deadly of the tropical diseases, malaria was the one disease that eighteenth and early nineteenth century colonists could expect to contract if they spent any significant time in the tropics. The toll from malaria and other tropical diseases was so deadly that West Africa earned the nickname "the white man's grave." Although contraction of malaria did not necessarily mean a death

sentence, the general debility from malarial fevers often resulted in increased susceptibility to other diseases.[9] Thus, to solve the puzzle of malaria was to significantly decrease the death rate of populations and troops in both temperate and tropical climates.[10]

The search for a cure for malaria followed Spanish conquistadors and Jesuit missionaries in South America as they entered the Amazonian jungles in search of indigenous peoples to convert to Christianity. Thus far, the two mainstays of *Plasmodium* treatment are derived from plants. Both *Cinchona* spp., as well as *Artemisia annua* were discovered by considering the traditional uses of medicinal plants. In the case of Cinchona in the 15th century the Spaniards Juan Fragoso and Nicolas Monardes [11] wrote the first known record about a malaria remedy that was much respected by the South American natives. They, in turn, passed it on to the Spaniards. South American Indians had used cinchona brews, which they called "quinas,"for fevers and other conditions [12,13] A later record came almost one hundred years later by Calancha of Lima (Peru), an Augustinian monk. He wrote in 1633 that a powder of quina, a Native American word meaning bark "given as a beverage, cures the fevers and tertians." [12] By 1643, the European medical literature also recorded the use of this New World fever remedy, which earned the name "Jesuit's bark" in the British apothecaries because of the importation and distribution of cinchona bark by the Jesuits whose missions extended from the Amazon to Patagonia.[12,13]

In the late 17th century, the famed physician Francesco Torti began using the bark prophylactically. He also insisted, unlike his contemporaries, in using high doses of the powdered bark swiftly and repeatedly at the first signs of malarial fevers.[14] His results eventually encouraged fellow physicians to follow his protocol. In 1820, Pelletier and Caventou, French chemist-pharmacists, isolated quinine out of the 30 + alkaloids in cinchona. This, coupled with the German chemist Sertürner's previous isolation of morphine from opium poppy (*Papaver somniferum* Papaveraceae**)** in 1805, profoundly shifted the direction of medicine to therapeutics based on single plant-derived chemicals,[15,16] the advent of the modern pharmaceutical industry and the use of pure compounds as the basis of most conventionally used medicines in industrial nations.

Beguilingly, quinine is but one of several active compounds in cinchona that is effective against malaria. In *Cinchona* spp. (Rubiaceae) there are at least 7 alkaloids, as well as other groups of constituents that contribute to the antimalarial activity.[17] (see Table 1) *Cinchona officinalis* bark contains up to 7% alkaloid content (by dry weight), with about 48% of that being quinine and derivatives of the cinchonine group, which also contain antimalarial properties.[16,18]

During World War II the United States military experimented with a mixture of cinchona alkaloids named totaquine.[19] Totaquine was defined as containing 7 – 12% anhydrous quinine, and 70 – 80% of total anhydrous crystallizable cinchona alkaloids. Thus, totaquine was a mixture of cinchona alkaloids which was easy to produce, even with low grade cinchona bark (low quinine content), and could have been a relatively inexpensive drug. The military concluded that totaquine was as effective as quinine in terminating acute attacks of malaria, but had a slightly higher rate of nausea and blurred vision. However, they also found that the 2 alkaloids cinchonine and cinchonidine were less toxic than quinine. A more recent study done with a mixture of three of the chinchona alkaloids, quinine, quinidine, and cinchonine, demonstrated a synergic effect against a culture of *P. falciparum*.[20] Additionally, the *Plasmodium* strains that

were resistant to quinine were up to 10 times more susceptible to the alkaloid mixture than any of the single alkaloids. It is possible that *Plasmodium* resistance could be at least delayed, if not avoided, with the prudent use of such therapeutic mixtures. (Table 1 shows overview of cinchona alkaloid activity.) Unfortunately, research to support such combinations are only recently being pursued and reconsidered.

Compound	Type of compound	Pharmacological action
3-alpha-17-beta-Cinchophylline	Alkaloid	Cytotoxic
3-beta-17-beta-Cinchophylline	Alkaloid	Cytotoxic
Avicularin	Flavonoid	Aldose-Reductase-Inhibitor
Cinchonidine	Alkaloid	Antimalarial Antipyretic
Cinchonine	Alkaloid	Antimalarial Antipyretic MDR-Inhibitor Synergist
Hydroquinidine	Alkaloid	Antimalarial
Quinidine	Alkaloid	Antimalarial Antipyretic MDR-Inhibitor
Quinine	Alkaloid	Antimalarial
Eupatorin	Flavonoid	Antimalarial
Oleanolic acid		Antimalarial
Quercetin	Flavonoid	Antimalarial
Tannins	Tannin	Antihepatotoxic

Table 1. *Cinchona* spp. compounds active against malaria
Source: Duke JA. (2006). Dr. Duke's Phytochemical and Ethnobotanical Databases. (JA Duke, ed.), Vol. 2006. http://www.ars-grin.gov/duke/.

Modern treatment of malaria

Today, even in severe manifestations of falciparum malaria, quinine continues to be a viable remedy for malaria and continues to be used in combination with other malarial drugs to inhibit the development of resistant strains of falciparum.[21] However, multi-drug resistance has become a leading obstacle to curing malaria and protecting against infection.[22] As a result, many researchers are calling for combinations of antimalarial drugs to prevent *Plasmodium* spp. resistance. One such example is artemisinin-combination therapies (ACT), designed to attenuate resistance. ACT is now recommended by WHO as the first-line treatment for uncomplicated malaria.[23] Medicinal plants are obvious multi-component remedies. For example, sweet Annie (*Artemisia annua*, Asteraceae), the source of artemisinin, contains at least 9 different antimalarial compounds (see Table 2).

Rath et al[24] have shown that artemisinin, a hydrophobic sesquiterpene lactone, is absorbed faster in humans from a tea preparation of the traditional Chinese medicinal plant sweet Annie than from tablets of pure artemisinin. This appears to be due to the co-occuring plant

constituents which appear to generate a high extraction efficiency of the lipophilic artemisinin in boiling water.[24] Moreover, mice fed dried *A. annua* plant material had about 40 times more artemisinin in their bloodstream than mice that were fed a corresponding amount of pure drug.[25] Notably, this amount exceeded by eight fold the minimum concentration of serum artemisinin (10 mg/L) required against *P. falciparum*.[26] A 2012 study found that whole plant treatment of *A. annua* is a more efficient delivery mechanism than the purified drug, thus reducing cost and improving efficiency.[25]

Antihepatotoxic	oleanolic-acid ; quercetin ; scoparone ; scopoletin
Antimalarial	artemetin ; artemisinin ; ascaridole ; casticin ; chrysosplenetin ; chrysosplenol-d ; cirsilineol ; eupatorin ; oleanolic-acid ; quercetin
Antiplasmodial	chrysosplenetin ; chrysosplenol-d ; oleanolic-acid ; quercetin
Antipyretic	α-bisabolol ; borneol ; menthol
Antiseptic	1,8-cineole ; α -bisabolol ; α -terpineol ; arteannuin-b ; β-pinene ; camphor ; carvacrol ; carvone ; geraniol ; kaempferol ; limonene ; linalool ; menthol ; oleanolic-acid ; rhamnocitrin ; scopoletin ; terpinen-4-ol ; thymol
Hepatoprotective	borneol ; isorhamnetin ; kaempferol ; luteolin ; oleanolic-acid ; quercetin ; rhamnetin ; scoparone ; scopoletin
Immunostimulant	astragalin ; coumarin ; eupatorin
Larvicide	cuminaldehyde ; linalool ; thymol
MDR-Inhibitor	artemisinin ; chrysosplenetin ; chrysosplenol-d
Parasiticide	artemetin ; casticin ; chrysosplenetin ; chrysosplenol-d ; cirsilineol ; eupatorin
Protisticide	α -bisabolol ; artemetin ; casticin ; chrysosplenetin ; kaempferol

Table 2. *Artemisia annua* Against Malaria from [27] Spelman, Duke, Bogenschutz-Godwin. 2006. CRC Press.

Not only do constituents of the plant appear to improve pharmacokinetic parameters *A. annua* contains at least 9 other compounds that contain antimalarial activity[27] (see Table 2). At least two of the flavonoids of *A. annua* appear to potentiate the mode of activity of artemesinin.[28] Two polymethoxyflavones, casticin and artemitin, although inactive against the malaria-causing protozoa, *Plasmodium* spp., have been found utilizing *in vitro* models to selectively enhance the activity of artemisinin against *P. falciparum*.[29] Two additional flavones that show very little direct growth inhibitory activity, chrysosplenol and chrysoplenetin-D, appear to be targets for the P-glycoprotein pumps known as multi-drug resistance (MDR) efflux inhibitors.[30] This provides further possible potentiation of artemisinin against malaria.[27] Considering that resistance of *P. falciparum* to mefloquine and structurally related drugs has been found to be due to the P-glycoprotein pump[31,32] this is particularly notable. Another trial showed that oil-based capsules of *A. annua* cleared parasites and fever more rapidly than did chloroquine, and by day 30, 92% of those subjects given a course of capsules for 6 days had no recrudescence (renewed activity of the parasite).[33]

Although *Plasmodium* recurrence in some human trials appears to be an issue using various extracts of *A. annua,* [33,34] the recrudescence issue could likely be addressed by a different dosing strategy or extraction method. There are positive studies, at least in the short

term, to support the use of an *A. annua* tea for the treatment of malaria.[34,35] In addition, Wilcox[36] reports on Chinese studies performed with ethanol extracts that reported better outcomes than those studies using the teas. The recrudescence rate with the use of tea of *A. annua* is likely due to the short half-life of artemisinin which does not kill all stages of *Plasmodium.* This is of concern because recrudescence is a risk for resistance. On the other hand, de Ridder et al[37] comment that *A. annua's* traditional use in China for 2000 years for fevers is apparently without the emergence of resistance.

Unfortunately, but predictably, there are reports of *in vitro* resistance of *Plasmodium* spp. to artemisinin derivatives [38,39] as well as reports of recrudescence in patients treated with artemisinin derivatives.[40] This is of particular concern due to the increase in demand of artemisinin derived drugs, from 22,000 treatment courses in 2001 to an estimated 200 million in 2008. Many researchers and organizations, including the World Health Organization (WHO), are calling for artemisinin combination therapies to prevent *Plasmodium* spp. resistance.[41,42] Single agents generate substantial evolutionary pressure on *Plasmodium* and generate the development of resistance and as such, combination therapies or "cocktails" prevent, or at least delay resistance. Unfortunately, the combination of only 2 antimalarials may not generate enough of a molecular challenge to prevent resistance. Recent reports from southern Cambodia report failure of the artesunate-mefloquine combination therapy.[43]

The recrudescence rate could likely be reduced further by 1. Extending the treatment period and/or 2. adding one of the low cost anti-malarials (e.g. chloroquine) that would be potentiated by the MDR inhibition of the flavonoids in *A. annua* or 3. adding another active medicinal plant with active constituents that have a longer half-life in combination with the *A. annua* treatment.

Willcox points out that there are 1277 plant species from 160 families listed that have been used to treat malaria. Unfortunately of these, five were listed as "endangered," thirteen were listed as "vulnerable," and three were listed as "near threatened." [44] In northeast India 65 medicinal plants from 38 different families have been reported to treat malaria,[45] while in South Viet Nam, of 49 plants identified as traditionally used for malaria, forty-six showed in vitro activity at 10 µg/mL.[46] Approximately 64% of the traditional malaria remedies in Kenya have been found in an *in vitro* model to exhibit anti-plasmodial activity.[47] Mills has pointed out that a source of empirical evidence for medicinal plant activity is provided by societies socially and geographically distant from one another finding common usage of the same genera.[48] For example, of the 1277 plants Willcox[44] lists, 47 species are used on 2 continents and 11 species on all 3 tropical continents are used as antipyretics or antimalarials. The plants used on more than one continent for the treatment of malaria could provide an informed beginning of searching for effective antimalarials, whether they are low cost traditional remedies or high-tech combination cocktails made from isolates. An initial study examining the use of plants used on 2 continents for parasitic infections confirmed activity.[49]

Notable mentions of medicinal plants besides the previously mentioned *Cinchona* spp. and *A. annua*, include the Ugandan formula "AM" in which 55% of patients had ♦adequate clinical responses and 8% had clearance of parasites.[50] *Terraplis interretis* showed high rates of adequate clinical response to the point of clinical cure.[44] Additionally, *Cryptolepsis sanguinolenta* (Asclepiadaceae) has demonstrated activity roughly equal to that of chloroquine; *Cryptolepsis* cleared fever 12 hours faster, and cleared parasites within 24 hr.[44] *Bidens pilosa* (Asteraceae) has shown activity against drug resistant *P. falicparum* parasites *in vitro* and *in vivo* in rodents. *Strychnopsis thouarsii* appears to be useful for prevention of malaria due to activity against the hepatic stage of *Plasmodium*.[51]

Studies with plants traditionally used for malaria treatment from various parts of the world (Vietnam, South Africa, and São Tomé and Príncipe) have intriguingly shown inhibitory activities against chloroquine sensitive or resistant strains of *P. falciparum*.[52] Worthy of further research, medicinal plant extracts demonstrating activity against chloroquine sensitive and resistant strains of *P. falciparum* include *Coscinium fenestra* (Menispermaceae), *Psidium guajava* (Mytraceae), *Vangueria infausta* (Rubiaceae), *Struchium spargano-phorum* (Asteraceae), *Cinchona succirubra*, *Tithonia diversifolia* (Asteraceae), *Cedrela odorata* (Meliaceae), and *Pycnanthus angolensis* (Myristicaceae).[53] Of the traditional remedies of Kenya including *Vernonia lasiopus, Rhamnus prinoides, Ficus sur*, some, such as *Vernonia brachycalyx and V. lasiopus* showed a stronger effect on resistant *Plasmodium* strains than the nonresistant strains.[47] *V. lasiopus,* which was found to potentiate chloroquine, also showed antiplasmodial activity comparable to *Cinchona.*[47]

Recent research on medicinal plants that have anti-plasmodial properties: Spilanthes acmella and Zanthoxylum chiloperone

Spilanthes acmella Murr. (Asteraceae; syn. *Blainvillea acmella* (L.) Philipson) is another plant from the traditional pharmacopoeia that is reported to be useful in the treatment of malaria. A related species, *S. oleracea* L., is a component of a formula known as Malarial-5, produced and sanctioned by the National Institute of Public Health in Mali for the treatment of malaria, relying primarily on ethnobotanical indications as evidence for treatment.[54]

Several bioactive compounds have thus far been elucidated from *S. acmella* which includes alkylamides and flavonoids. The *N*-alkylamides are fatty acid derivatives and have been identified in several species of *Spilanthes.* [55] Early work found spilanthol, also known as affinin or deca-2*E*,6*Z*,8*E*-trienoic acid isobutyl amide, a local anesthetic, as the main lipidic component [56]. More recent work has found acetylenic alkylamides such as undeca-2*E*-en-8,10-diynoic acid isobutylamide (UDA) in lower quantities. [57] However, these compounds and the extracts of *S. acmella*, have rarely been assessed for antiplasmodial activity.

♦ World Health Organization defines adequate clinical response as the absence of parasitaemia on day 14 or absence of fever (regardless of parasitaemia), without previously meeting the criteria for an early treatment failure.

Figure 1 illustrates the IC50s for the tested alkylamides in an investigation by Spelman and colleagues,[58] using spilanthol and UDA on *P. falciparum in vitro*. For the Brazilian mildly chloroquine sensitive strain PFB (Figure 1A), the IC50s for spilanthol and UDA are 16.5 and 41.4 µg/mL, respectively. While for the Thailanese chloroquine resistant strain K1 (Figure 1B), the effect of the alkylamides is significantly greater, with IC50s of 5.8 and 16.3 µg/mL, respectively.

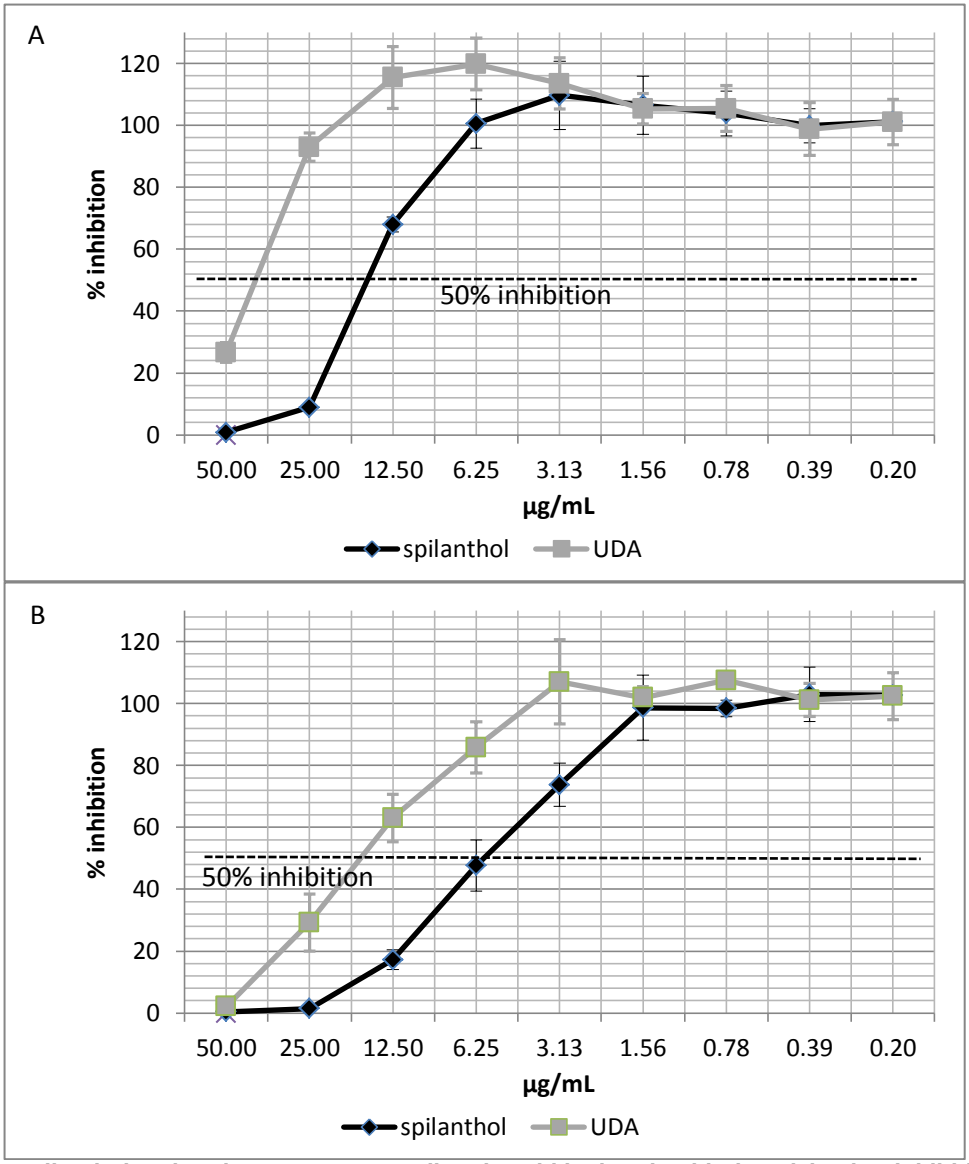

Figure 1. Spilanthol and undeca-2E-ene-8,10-diynoic acid isobutylamide (UDA) *in vitro* inhibition of *P. falciparum* strains.

1A. Spilanthol and UDA show IC50s on the *P. falciparum* strain PFB at 16.5 and 41.4 µg/mL.
1B. Spilanthol and UDA demonstrate IC50s of 5.8 and 16.3 µg/mL on the chloroquine resistant strain *P. falciparum* K1. Growth inhibition was determined by comparison of the radioactivity incorporated into the treated culture with that in control culture from the same plate. Chloroquine served as a positive control (IC50s: PFB – 28.4 nM; K1- 100 nM). Values are mean ± S.E.M. of experiments performed in triplicate. From #59: Spelman *et al.* 2011. *Phytother Res.* Jul 2011;25(7):1098-1101.

Further studies into *S. acmella* were performed *in vivo* on *P. yoellii yoelii*-infected mice using a whole plant galenic extract of 100% water (10 mg/mL),[58] as S*pilanthes* remedies are commonly prepared as tea. In addition, a fresh plant ethanolic extract (10 mg/mL, 70% ethanol final volume), due to these extracts containing ten times the concentrations of spilanthol as aqueous extracts, was also utilized [57]. As seen in Figure 2, the control group had an average parasitemia of 17.7% ± 3.3 five days after infection. A significant reduction of parasitemia by treatment with spilanthol (5 mg/kg) and *S. acmella* water extract (50 mg/kg) was observed with parasitemia decreased to 7.3% ± 1.4 (59% reduction) and 8.4% ± 1.7 (53% reduction), respectively ($p < 0.001$). The *Spilanthes acmella* ethanol extract (50 mg/kg) had less of an effect with an average parasitemia of 11.3% ± 2.0 (36% reduction) ($p = 0.01$).

The water extract and the isolated spilanthol exhibited the highest activity under the experimental conditions utilized. This suggests that in addition to spilanthol, there may be water soluble constituents that are also active against *Plasmodium*. In addition, common hydrophilic phytochemicals have shown potentiation of known antimalarials, [59] suggesting that there is likely multiple modes for the observed effect of *Spilanthes* extract. The treatments could also induce immunological activity contributing to a reduction in parasitemia. Recent studies on structurally similar alkylamides, and UDA specifically, report immunological activity at low (< 1.5 μM) concentrations. [60] Further investigations are necessary to determine the viability of this traditional medicine, and its lead compounds, for the treatment of malaria.

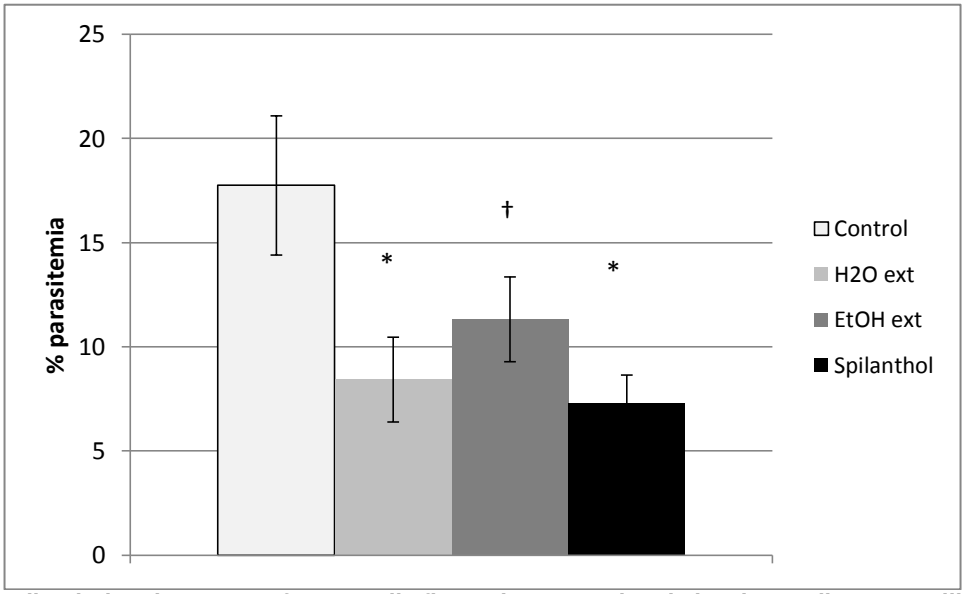

Figure 2. Spilanthol and extracts of *S. acmella flos* reduce parasitemia in *Plasmodium y. yoelli*-infection.

Spilanthol, water extract, or (70%) ethanol extract of *Spilanthes acmella* inhibit parasitemia as compared to the control group. Inoculation with *P. yoelli yoelli* 17XNL and treatment started 2 hours later. Treatments (spilanthol 5 mg/kg; water extract 50 mg/kg; ethanol extract 50 mg/kg) were given two times a day for four days. Parasitaemia was determined 5 days after infection by microscopic examination of Giemsa stained-thin blood smears. Values are mean ± S.E.M. (n = 5 for each group). † $p = 0.01$; *$p < 0.001$. From #59: Spelman *et al.* 2011. *Phytother Res.* Jul 2011;25(7):1098-1101.

Another promising plant is *Zanthoxylum chiloperone* var. *angustifolium* Engl. (syn. *Fagara chiloperone* Engl. Ex Chod. & Hassl.), Rutaceae, a diecious tree indigenous to the central and southern continent of South America, which is called "tembetary hu" and "mamicão." [61,62] A decoction of *Z. chiloperone* root and stem bark has been used in traditional medicine to treat malaria and for its emmenagogue and antirheumatic properties.[63,64] Studies have shown that the crude extract of the stem bark has activity against *Trypanosoma cruzi* [65] and antifungal activity *in vitro*.[66] Further investigations demonstrate that canthinone type alkaloids, canthine-6-one and 5-methoxycanthine-6-one (figure 3), are antifungal [66,67] and effective *in vivo* against *Leishmania amazonensis* and *Trypanosoma cruzi*. [64,65] Canthine-6-one has been suggested to be an inexpensive and safe treatment for use in long-term oral treatment as well as a good candidate against drug resistant strains of *T. cruzi.*

Other compounds have been isolated from species of *Zanthoxylum* including the pyranocoumarin avicennol and alkylamides (figure 3), such as the sanshools. [67-70] To date, there is a paucity of research on the biological activity of avicennol, which has been previously identified in *Z. elephantiasis* Macfad.[67] Recent work with avicennol reports an induction of UDP-glucuronosyltransferases (UGT), specifically UGT1A1, which detoxifies xenobiotics. [71]

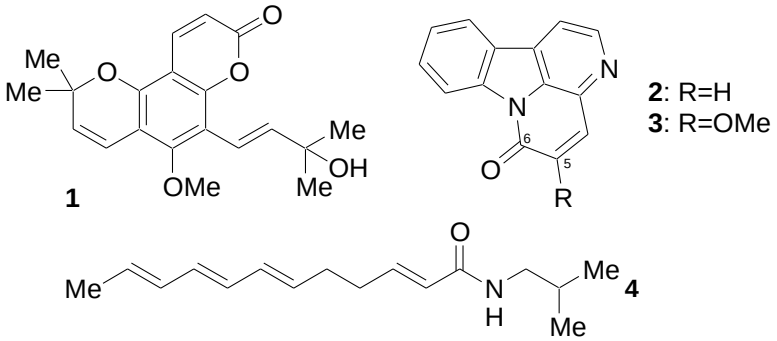

Figure 3. **Chemical structure of *trans*-avicennol (1), canthin-6-one (2), 5-methoxycanthin-6-one (3) and sanshool (represented as *Z*-isomer) (4).**

The alkylamides are also contained in *Zanthoxylum* spp.[72] And while many *Zanthoxylum* spp. are known to contain alkylamides, to the best of our knowledge there are no reports of the occurrence or identity of alkylamides in *Z. chiloperone*. Notably, these compounds have also been shown to be antiparastic, [73] as well as insecticidal.[74] Recent reports have shown *in vitro* and *in vivo* anti-plasmodial activity of alkylamides identical to, or similar to, the alkylamides occurring in other *Zanthoxylum* spp. [72]

Unfortunately, there has been limited exploration of *Zanthoxylum chiloperone'*s chemistry and biological activity. Cebrián-Torrejón and Spelman *et al.* [75] after isolation of *trans*-avicennol, canthin-6-one and 5-methoxycanthin-6-one , showed the half maximal inhibitory concentrations (IC50s) of extracts and the previously listed compounds. Figure 4 illustrates the IC50s for avicennol, canthine-6-one and 5-methoxycanthine-6-one on the *Plasmodium falciparum* strain F32. These data show that F32 is the most sensitive strain to avicennol and canthine-6-one (0.5 and 2.0 μg/mL, respectively). Further testing of additional *P. falciparum* strains demonstrated that these compounds all had approximately the same activity on K1, PFB and FcB1strains. Table 4 shows the results on parasite growth in tabulated form. Note that the crude extracts of *Z. chiloperone* demonstrated robust activity. This is possibly due to the

combination of the canthin-6-one type compounds and the pyranocoumarin. In addition, the alkylamide(s), previously shown to be active against *P. falciparum in vitro* and *P. yoelii yoelii in vivo*[72] may also exert antiplasmodium activity. Considering that traditional extracts of *Z. chiloperone* are used to treat Chagas disease, this is an intriguing finding and suggest that there may be various modes of *Plasmodium* inhibition due to the presence of multiple active compounds and possible other, unknown, compounds.

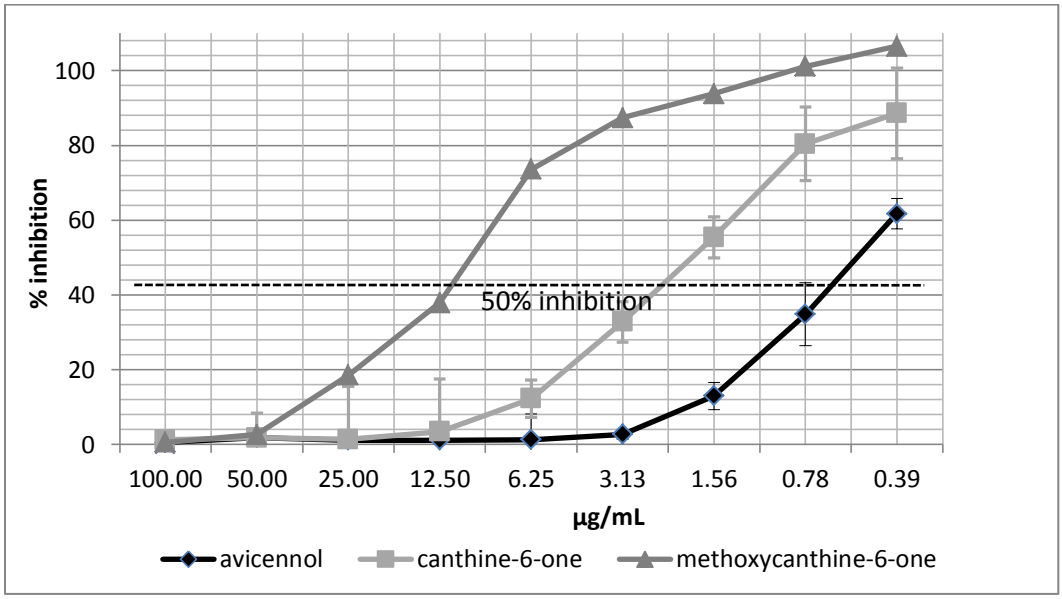

Figure 4. IC50s of Isolated Compounds from *Z. chiloperone*

Against *P. facliparum* F32, avicennol shows an IC50 of 0.5 µg/mL; canthin-6-one and methoxy-6-one show IC50s of 2.0 and 10.4, respectively.

IC50 values for the positive control chloroquine are also listed in Table 4. Note that the IC50s for the K1 clone of *P. falciparum* were considerably less than those published by Elueze *et al.* [76] and Fivelman *et al.* [77], but higher than Sharrock *et al.* [78].

	***P. falciparum* strain IC_{50}s (µg/mL)**			
Compounds	**F32**	**K1**	**PFB**	**FcB1**
Trans-avicennol (**1**)	0.5	2.7	1.2	2.2
Canthin-6-one (**2**)	2.0	5.3	3.2	4.0
5-methoxy-6-one (**3**)	10.4	5.1	*nt*	*Nt*
Extracts				
DCM	8.9	8.9	*nt*	*Nt*
EtOH	10.5	9.3	*nt*	*Nt*
MeOH	89.5	>100	*nt*	*Nt*
Positive Control				
Chloroquine	*2.6	*77.4	*27.8	*62.8

*Chloroquine concentrations are in nM
nt – not tested

Table 4. IC_{50} of *Z. chiloperone* Isolated Compounds and Extracts.

Cebrián-Torrejón *et al.* [75] confirmed that the antimalarial activities measured by [^{3}H]-hypoxanthine incorporation were due to the intrinsic antiparasite property of the experimental compounds and not due to hemolytic activity. They reported that *trans*-avicennol, canthine-6-one and 5-methoxycanthine-6-one, as well as the dichloromethane, ethanol and methanol extracts of *Z. chiloperone* had no haemolytic effect on erythrocytes.

Table 5. Cell Survival of MCR5 Cells Incubated with *Z. chiloperone* Isolated Compounds and Extracts.

Experimental compound(s)	IC_{50} (μg/mL)
Trans-avicennol (**1**)	4.4
Canthine-6-one (**2**)	9.4
5-Methoxycanthine-6-one (**3**)	12.1
DCM extract	12.3
EtOH extract	13.0
MeOH extract	>100

DCM - dichloromethane; EtOH - ethanol, MeOH – methanol

Cebrián-Torrejón *et al.* [75] also probed the cytotoxicity of the before mentioned compounds and extracts using MRC5 cells. Table 5 shows the results of these assays. *Trans*-avicennol had the most cytotoxic effect on MRC5 cells at an IC50 of 4.4 μg/mL. The canthinone alkaloids, canthine-6-one and 5-methoxycanthine-6-one, were observed to have IC50s at 12.1 and 12.3 μg/mL, respectively. Supporting the frequent use of this plant species in traditional medicine, the crude extracts demonstrated less cytoxicity with the DCM extract and the ethanol extract showing 12.5 and 13.0 μg/mL IC50s, respectively. The hydrophilic methanol extract had no detectable effect on cell survival up to 100 μg/mL.

Organizations of interest

Despite the prevalent use of traditional remedies, with or without pharmaceuticals, there appears to be few organizations, dedicated to researching medicinal plant species as home remedies or drug-leads to treat *Plasmodium* spp. infections. Exceptional organizations such as The Research Initiative on Traditional Antimalarial Methods (RITAM), Doctors for Life, ICIPE and the Plant Medicine Innovation Group have dedicated their energies towards the political, economic and research efforts of medicinal plants and other issues related to health and malaria. Many of these researchers believe that medicinal plants have the potential of solving the medical and societal issue of multi-drug resistance.[79-85]

An organization that deserves particular mention is Action for Natural Medicine International (Anamed International). Since 1998 Anamed International has traveled to over 75 countries distributing 1,200 *A. annua* "starter-kits" (containing seeds and instructions for their use). Further activities include organizing over 100 week-long training seminars on natural medicine in 20 different countries, the majority in Africa.[86] Local participants of these seminars, eventually continue to run week-long training seminars themselves. These seminars are primarily focused on the cultivation and use of *A. annua* and the full details are available in Anamed publications.[87,88] The preparation recommended is an infusion of 5 g of dried A. annua leaf

powder in 1 litre of water, to be taken as 250 ml four times a day (for adults). A published study consisting of retrospective surveys suggest they are saving lives.[89]

Is there are case for working with simple home-grown remedies?

As physicians are suggesting combinations of antimalarial drugs to prevent *Plasmodium* spp. resistance,[41,42] the esteemed ethnobotanist James A. Duke, a veteran of malaria ridden areas in Latin America, suggests that rather than increasing the costs of malaria treatment using multiple drugs, the use of the tea or ethanolic extract of *A. annua* with its 9 different antimalarial compounds might prove as efficacious.[27,90] Duke's suggestion that extracts of *A. annua* can be used as a "cocktail" therapy does have support by the previously mentioned clinical trials on *A. annua* and the above mentioned retrospective report. This could lead to self-reliance therapy that is readily available to impoverished areas where the death rates from malaria are high.

Treatment cost and income are important variables affecting the choice of malaria treatment and drug resistance. The majority of malaria-ridden countries spend less than US $10.00 per capita annually on health, creating a situation where a 50 cents becomes a prohibitive cost of treatment.[3] Perhaps partially due to such economics the recommended treatment in many high-transmission areas are antimalarial drugs (i.e., chloroquine or sulfadoxine-pyrimethamin) that are partially or completely ineffective. [91]As a result of cost and lack of access to health care facilities, medicinal plant preparations remain a popular choice for the rural poor.[92] Studies report up to 75% of African patients with malaria use medicinal plants,[93] while in French Guiana 33% report regularly use of herbal remedies to prevent febrile illnesses and malaria.[93] Mothers in rural Africa commonly start malaria treatment of their children with herbal therapies before they initiate pharmaceutical treatment.[94]

Since the treatment of malaria by the poor often involves buying whatever they can afford and not necessarily the correct dosage for effective treatment of an episode, thus creating resistance, it could be that new pricey pharmaceuticals *combined with* properly used medicinal plant preparations would stave off resistance to the pharmaceuticals. A more economic strategy may be using the old pharmaceutical antimalarials with medicinal plant preparations. Although this may initially seem irrational to those unaware of the research on medicinal plants and malaria, considering that recent treatment strategies to reduce the emergence of de novo resistance relied on antimalarial drug combinations,[91] it follows that if a plant contains compounds that are antimalarial (and as previously stated the known antimalarial plants commonly have multiple antimalarial compounds), then a combination of the properly-dosed medicinal plant extract with an inexpensive pharmaceutical antimalarial may greatly facilitate elimination of the malarial parasite. If a medicinal plant is extracted and dosed properly it may be a viable "combination" treatment.

Likewise, considering the number of plant extracts that have shown activity against *Plasmodium* spp., the rich tradition of treating malaria with medicinal plants and the research suggesting promise of many of these remedies, it seems unlikely that there would not be more species that could explored. Given that effective medicinal plant extracts could shift the benefit:cost ratio from dollars to pennies, and that many known antimalarial plants, including *A. annua,* grow prolifically in tropical equatorial climates, this could significantly change the

societal and economic burden of disease in many parts of the world. In addition, properly planned cottage industries of producing plant based remedies for the treatment of malaria and other disorders could generate income for rural communities. Nevertheless, until enough resources are marked for allowing research on the potential of medicinal plants as a low cost, easily accessible solution, this potential may never be known. If political and economic issues are removed from the labyrinth of malaria treatment, that medicinal plants, often readily available and affordable as opposed to pharmaceuticals, may provide at least a partial solution to one of the planet's leading causes of mortality.

The use of local plant medicines offers greater social acceptance, consistent supply, and the opportunity to support local economic activity. Is it possible that dosing with such inexpensive remedies may provide partial protection without stirring an increase in resistant strains? Although some non-governmental organizations (NGOs), such as anamed, have been using artemisia tea for many years in countries like PR Congo there has unfortunately been no structured records of these initiatives. It is important that there be rigorous research of such prospects for a pragmatic, even if partial and temporary, solution for African populations facing malaria. In March 2006 a group of NGOs sponsored a meeting in Nairobi, Kenya. The meeting called among other measures for support of village level clinical trials in Africa to monitor the safety and efficacy of artemisia tea and other local whole herb preparations. A follow-up discussion has taken place with the Prince of Wales Foundation for Integrated Health to explore ways in which further research may be supported.[95]

There is evidence that multi-constituents of a plant can have synergistic effects. *Artemisia annua* appears to be more than artemisinin. As previously mentioned, the plant includes at least 9 other compounds that contain antimalarial activity. Some of *Artemisia's* flavonoids potentiate artemesinin. Two polymethoxyflavones, casticin and artemitin, although inactive against *Plasmodia* alone, have been found to selectively enhance the activity of artemisinin against *P. falciparum*. Furthermore, two additional flavones that show very little direct inhibitory activity, chrysosplenol-D and chrysoplenetin, appear to be MDR efflux inhibitors that could potentiate artemisinin. Similar observations have been made on the plant source of quinine: following the positive research by the US military on the cinchona alkaloidal mixture totaquine in the treatment of malaria, a synergic effect of a mix of three chinchona alkaloids, quinine, quinidine, and cinchonine has been demonstrated against a culture of P. falciparum.[95]

The Way Ahead

With the advent of climate change, it is likely that malaria will once again occur in non-tropical climates.[96-99] Further spread of this major disease suggest that all means possible should be considered to eliminate, control and treat malaria. Unfortunately, current politics has created a paucity of information to guide policy. There are clear ways to improve available information in order to make informed policies:[95]

1. Improved collation of reports from current field studies in the use of herbs.

2. Review prospects of herb use as
    a. primary treatment in remote areas,
    b. prophylaxis against malaria,

c. complements to other antimalarial treatment.

3. A meeting of key experts to set up the most appropriate research models, leading to,

4. well-planned and rigorous clinical trials to determine the efficacy and effective dosage of artemisia tea and other medicinal plant speciess

Conclusion

Comprehensive evaluations of medicinal plants are urgently needed before more plant species are lost and knowledge of specific traditional medicines becomes irretrievable. While the study of a medicinal plant and its many components—some of them unidentified or having unknown properties—is theoretically, economically, and technically challenging, it should not be abandoned for sake of investigative expediency. Research into the multi-component nature of medicinal plant remedies offers a segue way into more complex therapeutics.[34] Thus, the issue of using herbal remedies to alleviate human suffering is not one of merely assessing efficacy and safety,[1] but a matter of the medical community's struggle to understand a pharmacological paradigm that embraces the complexity of bio-molecular networks using multi-component extracts such as medicinal plants. With modest investment the potential benefits for the human struggle with malaria are dramatic.[95]

Implementation of multi-component pharmacological models (e.g. network pharmacology), which would lead to more complex therapeutic agents, could result in delayed antimicrobial resistance, decreased infectious morbidity, and less healthcare expenditures. But certain challenges have held drug therapeutics in the simplistic model that encourages the search for silver bullets. One obstacle, a limited collection of analytic tools, has been solved with the newest generation of high-tech analytical tools. Microarrays and related technologies are now economically feasible to the point that running hundreds of arrays are possible. Such an approach will demand more statistical, mathematically, and computational prowess. But if successful, this could generate improved therapeutics based on patient specific treatments and dietary guidelines, resulting in less human suffering and decreased economic burden. A second obstacle, a clashing of philosophies, is in the process of resolving. Ohno and collegues suggest that further progress will be made when all parties involved give up their subjective certainty and allow unbiased and more methodologically relevant investigations of medicinal plant species.[37]

After a hundred years of technological innovation, plants are still the primary source of leads for pharmacologically active compounds. The United Nations Convention on Biological Diversity takes the noteworthy stance that evolution has been selecting and perfecting diverse bioactive molecules for millions of years.[103] The further development of the science of pharmacology is likely to grow considerably beyond the current tenants of isolation, selectivity and potency if it takes a cue from the 300 million years of plant evolution that have perfected a complex chemical means of defense against microbes and other predators. The study of phytochemical defense offers an opportunity to expand the foundational philosophy and techniques of the search for new drugs: They may best be utilized, not as expensively manufactured silver bullets hitting a single target, but as multi-component, broad-spectrum,

pleiotropic molecular cocktails interfacing with cellular networks. This natural technology has been harnessed by traditional cultures for many centuries.

Unfortunately, the current prejudice of healthcare professionals who were not exposed to a curriculum introducing principles of network pharmacology and the understanding of herbal medicines as more than complex agents masking a single active constituent makes medicinal plants difficult to comprehend. Nonetheless, it is a scientific imperative for the progress of medicine that the time-tested methods of traditional medicine and the hi-tech modern pharmaceutical approaches coalesce. As Bodeker and Wilcox[100] suggest, if safe and effective antimalarial preparations could be produced inexpensively from indigenous flora, such remedies could become an added tool, especially in areas that make modern pharmaceuticals inaccessible, to the efforts of dealing with a disease that is a leading global killer. However, it is first necessary that proponents of medicinal plants, as well as the promoters of conventional pharmaceuticals, relinquish their subjective certainties. Both traditional and conventional healthcare systems seek to alleviate human suffering, both systems have merit, and both systems provide therapeutic options. All parties must learn to stretch pharmacological principles, beyond simplistic modeling and economic gain, to therapeutics based on improving the human condition. We must not let prejudice against therapeutics that are complex and not fully understood impede the use of life-saving remedies. Furthermore, where plant species intersect with medicine, we must keep an eye towards species preservation, sustainability, and the ethics of interfacing with traditional cultures.

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