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Opinion

Towards Making the Real Antidote for Malaria

Anthony M Kyriakopoulos[1]*, Stella Balliou[2], Nikolas Khoury[2] and Vasillios Zoumpourlis[2]

[1]Nasco AD Biotechnology Laboratory, Pireus, Greece

[2]National Hellenic Research Foundation, Athens, Greece

***Corresponding Author**: Anthony M Kyriakopoulos, Nasco AD Biotechnology Laboratory, Pireus, Greece.

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Hellenic Philosopher Heraclitus (600BC) described the nature's law of the opposites. In medicine this is observed by the disease / antidote relation. It seems that Artemisinin, the so-called antidote for curing malaria, being produced by nature, is a good example for the law of the opposites. Ge Hong (284-363 AC), was a Chinese medical physician who depending on his writings made Professor Tu Youyou and her colleagues back in early 70's able to focus on the discovery of the antidote made by nature to cure malaria from Qinghao.

What is Ching-Hao and Qinghao?

Ching-Hao in Chinese traditional medicine refers to a group of medicinal recipes that cure malaria [1]. These recipes involve more than one species of *Artemisia* (or *Artemesia*, a word deriving from the Hellenic Goddess Artemis, the sister of Apollo). It is indeed rather difficult to distinguish through ching-hao, the medicinal properties of the Artemisia species devoted to cure malaria as recorded over thousands of years by traditional Chinese physicians. Qing-hao refers to the color (qing – dark green), of the leaves of the plant that are used to make the traditional medicinal preparation. These plants may be or may not be the *Artemisia annua*. However, *Artemissia annua* together with *Artemisia apiacea* and *Artemisia lanceolata* are the only known species found to produce sufficient quantities of the specific sesquiterpene lactone with the unusual peroxide bridge called artemisinin.

The Pharmacognosy of Artemisinin.

Artemisinin molecule is not soluble in either water or ether. Professor Tu Youyou and her team after many attempts, and only when following Ge Hongs recommendation to soak the entire plant material in water resulted to the initial ether extract with which malaria was treated successfully in mice in early 70's. Thereafter other *A. annua* extracts cured 90 % of falciparum and vivax malaria patients [2]. Thus, the initial ether extract mixture should have contained high amounts of artemisinin together with other components of the plant juice like flavonoids that may synergistically kill the high parasitic load during acute phases of malaria [3]. During the preparation of ether extract, Professor Tu Youyou has also been unfortunately omitted to have included in the methodology another crucial recommendation of Ge Hong. This was to wring out, in other words to squeeze out, the entire contents of the soaked plant material as used to do in ancient times [4]. In this way, even at that time it may have been known that other important synergistic activities from the plant material have worked as anti-malarial [5]. Since that time, it has been well documented that flavanol structures work together with artisininin to form supra-molecular complexes and in a sense, provide positive pharmaceutical efficacy of artemisinin's antimalarial activity through its binding ability with the iron of hemin [6]. However, it is also known that other *Artemisia* species have anti-malarial activity although that these do not contain artemisinin. For example, *Artemisia absinthium* and *Artemisia abrotanum* extracts have successfully treated malaria in Europe and their activity is attributable to other constituents of the plants. This is also the case for *Artemisia afra* were its anti-malarial activity is attributable to a complex mixture of flavonoids and sesquiterpene lactones, rather than to a single compound [7].

Even in *Artemisia annua*, the callus of the plant that does not contain artemisinin has some noticeable anti-malarial activity [8]. Amongst the 29 sesquiterpenes found in *A. annua*, notably, Arteannuin B. potentiates the effect of artemisinin [9]. In addition, from at least 36 flavonoids produced from *A. annua*, many have anti-malarial activity *in vitro* alone. Also, most importantly, these compounds show

synergy with artemisinin to kill parasites [10]. Chrysosplenol-D (the most abundant flavone in plant material) has the greatest potentiating effect to artemisinin's anti-malarial activity. Important for in the vivo activity of artemisinin is the catalytic role of these flavones that provide to the hemin - artemisinin interaction and also the aid they provide on artemisinin's solubility.

Further, regarding the bio-availability of pure artemisinin versus the plant derived artemisinin it has been documented that there is a comparatively high level of transfer of artemisinin into the bloodstream of mice when fed from plant material rather than by being fed with its pure form (where the level of delivered drug is undetectable) [11]. This has also been detected in human subjects drinking artemisinin by tea infusions [13]. In addition, when flavonoids are present together with sesquiterpene compounds there may be less chance of resistance occurring due to the combined action of multiple agents attacking pathogens simultaneously.

Insights to the original ether extract

The original ether extract investigated by Professor Tu Youyou, even with the improvement of soaking the entire plant in water as said by Ge Hong, took into account the extraction of polar fractions of the plant that may be soluble in water but less soluble in ether. For example, a salt that may be soluble in water may be less soluble in ether depending on its partition coefficient K:

Ksolute = Cether / Cwater.

Importantly, *Artemisia* family amongst medicinal plants has the highest content of potassium. Especially *Artemisia annua* has been shown to contain more potassium than any other species [12]. Flavonoids exist also in salts. These are called flavylium salts. At somewhere neutral pH and especially under high sodium or potassium salt concentration the production of these salts may be favored. Potassium may also react with artemisinin as this is more basic relative to its precursor dihydroartemisinic acid. This may be catalytic to the formation of even larger complexes (salts), of naturally existing flavonoids and artemisinin in order to give relative unstable flavylium - artemisinin complexes. Such supramolecular structures may have been observed by Bilia AR., *et al.* in 2002 [6]. The naturally existing supramolecular structures can be extremely valuable for artemisin's efficacy potentiation as they fasten binding of artemisinin with hemin that otherwise is a somewhat slow reaction. Similar reactions may account for all naturally existing sesquitrepenes and flavonoids in all *Artemisia* species. In a sense, what has been observed so far from all naturally deriving extracts may be the remains of some quantities of these flavylium - artemisinin salts or supramolecular structures that are more water soluble than artemisinin unaccompanied and may have survived through various extraction procedures. Complementary, it is rather unfortunate that hexane extraction for artemisinin used as a standard, omit's co-existing flavonoid and chalcone content of *A. annua* that is known to promote anti-parasitic and anti-cancer activities [5].

Extraction efficiencies of greater than 70 % have been noticed for artemisinin and these are made possible due to the enhancement of otherwise low water solubility of artemisinin by the action of other constituents present in *Artemisia annua* [13]. However, this field remains without any thorough investigation. Finally, it must be noted that sesquiterpene lactones especially the artemisinin with its unusual peroxide ring are reactive esters sustaining reversible hydrolysis, reduction, and aminolysis reactions. These intermediate molecules may also contribute to anti-malarial properties if present as parts of a given extract, or also according to their solubility may not be present to confer any anti-parasitic effect.

Stabilization technology related to artemisinin and synergistic flavones

Special attention must be made on the property of lactone formation called halolactonization where a halogen attacks the alkene and the halogen is captured intramolecularly via an electrophilic addition by the adjacent carboxylic acid. Regarding artemisinin biosynthesis in the plant [14], its precursor molecule, dihydroartemisinic acid has an open ring thus making extremely feasible the halolactonization event under the presence of chloride and bromide anions. Complementary, the polymethoxyflavones, namely casticin, artemetin, chrysosplenetin, chrysosplenol-D and circilineol present in *Artemisia annua*, can also be stabilized as flavylium salts under the presence of potassium – halogen salts at neutral pH.

Aqueous alkaline extraction medicinal preparation as described in Kyriakopoulos AM, Dinda B. 2015 [15], may thus open new ways towards the pharmaceutical development of the whole of naturally derived phytochemical complexes within Artemisia species to effectively cure malaria.

In 1973 Professor Tu Youyou and her team has synthesized dihydroartemisinin, the water soluble form of artemisinin that proved to be even more active than the original artemisinin molecule found in *A. annua* [2]. Thereafter, Artesunate, artemether and other compounds were synthesized in order to overcome dihydroartemisin deficiency. Unfortunately, this evidently led to their use as monotherapy that exerted a selective pressure for resistant strains of *Plasmodium* spp. to emerge [16]. Since 2006 WHO briefing, artesunate and artemether where recommended to form the core of new artemisinin-based combination treatments (ACT) for malaria in order to prevent the ever spreading resistance [17]. However, the built in chemical instability of artemisinin and related compounds and the proneness of dihydroartemisinin to an accelerating breakdown of inactive derivatives, especially under tropical climatic conditions, determine their biological activity [18] and this may be a central cause of alarming multi resistance emergence nowadays [19]. Newer semisynthetic approaches of artemisinin are under development but their effectiveness remains to be seen [14].

The question that needs to be answered quickly is whether all these approaches are mostly dogmatic to the older fashion of drug development. This dogma says to provide a single or a mixture of synthesized (profitable to the market) compounds rather than mimicking nature to approach towards the real antidote and provide a cure for malaria. In the meantime, millions of patients are in danger as parasite's multi-resistance is spreading.

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