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Tannins in Artemisia: the hidden treasure of prophylaxis

Opinion

Proanthocyanidins (condensed tannins) are oligomeric and polymeric products of the flavonoid biosynthetic pathway. They are present in the fruits, bark, leaves and seeds of many plants, wine and teas, and are increasingly recognized as having beneficial effects on human health. They have attracted little interest in malaria research because *in vitro* they show no significant antimalarial activity. Anthocyanins and pro-anthocyanidins are responsible for the beautiful color of leaves in autumn and for the colors of fruits. The major sources of proanthocyanidins in the American diet are apples (32%), chocolate (18%) and grapes (18%) There are not many data in the literature on the content of pro-anthocyanidins in food and plants, probably because of the difficulties to analyze them correctly. A rather complete table has been published by the US Department of Agriculture.[1]

For fruits and berries the highest content in mg/100g is found in blueberries (331), cranberries (418), blackberries (418), apples (120), plums (256) but very little in bananas, nectarines, mangos, peaches, pears, cherries, raspberries. In cereals and beans the leader by far is sorghum sumac (1900), various beans are around (500), but only small contents are found in barley and peas. In nuts and seeds, grape seed is the leader (3 500), hazelnuts are following with (500), pistachios (237), almonds (184), but only traces in peanuts, cashews. In spices the content is often very high: cinnamon (8100). It is not only the average content which is important, but the degree of polymerization may vary strongly from plant to plant: 1.61 for *Trifolium repens*, 3,57 for *Pinus radiates* needles, 7.39 for *Lotus pedonculus*, 8.1 for grape seeds, 34.9 for grape skin.[2] In sorgho the degree of polymerization is higher than 10. In cranberries dimers and trimers predominate. It is very difficult to find studies on pro-anthocyanidins in Artemisia plants. In *Artemisia afra* very high concentrations were found: 1 990 mg/100g.[3] A recent paper deals with *Artemisia herba alba* and finds a concentration of 2100mg/100g.[4]

Another paper detected the presence of anthocyanidins and tannins in several Artemisia species in Iran without quantifying them: *A. absinthium, A.annua, A.biennis, A.diffusa, A.santolina, A turanica, A.vulgaris, A. sieberi*.[5] A more recent paper from Iran finds 340mg/100g in *Artemisia annua* leaves and only 30mg/100g in stems.[6] A paper from Turkey finds an average of 420mg/100g of tannins in *Artemisia absinthium*. A study from Algeria compared the total extractable tannin content in some browse plant species. For Graminae they find an average of 600mg/100g DW, for Fabiaceae 400, for *Artemisia campestris* 5700 and for *A.herba alba* 3600mg/100g.[7] Tannin in *A absinthium* varies significantly from one region in Tunisia to another, from 40 to 150mg/g.[8] A study from Cameroon confirms that Artemisia plants are very rich in tannins.[9] A recent analysis from Algeria finds an average of 2% proanthocyanidins in different solvents for *Artemisia herba alba*.[10] Another study finds 11% fo*r Artemisia campestris* and *Artemisia herba alba*.[11] And another paper 8% for *Artemisia absinthium*.[12]

The big spread in analytical data of course is due to different analytical methods. A standardized technique needs to be defined and implemented. Sodium bicarbonate enhances the extraction of tannins from medicinal herbs. The feature is used in Turkish tea houses. Some bicarbonate is added to the water used for decoction and the infusion becomes darker.[13] Medicinal herbs, at least those with antimalarial properties, like *Cymbopogon citratus* (lemongrass) *Vaccinium macrocarpon* (cranberry), *Aziradichta indica* (neem), *Cinnamomum verum* (cinnamon) generally contain proanthocyanidins. An analysis of 11 plants in Brazil which are spontaneously used as medicinal plants reveals that their total tannin content is always high and ranges from 6800 to 13000mg/100gDW. Plants like *Moringa oleifera* or *Panax ginseng* who do not contain condensed tannins have no antimalarial properties.[14] Artemisia plants and/or proanthocyanidins are known to decrease serum uric acid levels.[15] All this would mean that Artemisia plants are very high in condensed tannins. A property which has been ignored and deserves further study. But why have they been ignored? The reason may be simple: 95% of the Artemisia extracts of Artemisia plants in the research papers are obtained with organic solvents, and tannins are insoluble in organic solvents like chloroform, hexane, benzene, ether and are sparingly soluble in ethyl acetate. The polymeric nature of proanthocyanidins makes their analysis and estimation difficult. For this reason, little is known about their consumption, although they likely contribute a large part of the daily polyphenol intake.

The antioxidant properties of plants are to a large extent related to their content in proanthocyanidins. Their antioxidant capacities are higher or comparable to vitamin C or E.[16] Antioxidant activities depend on their configuration and degree of polymerization. In the aqueous phase, they increase from monomer to trimer and decrease from trimer to tetramer. Galloylation and glycolysation decrease the activity.[17] The Iranian study (M Mojarab op.cit) found for the methanolic extract of 14 different Artemisia species a positive correlation between anthocyanidin content and antioxidant activity by the TBA method. Among all *A.sieberi* has the highest activity. There are other studies which show that *Artemisia annua* has a lower antioxidant power than other plants of the family, like *A ludoviciana*.[18] Concentrations found in polar solvents (water, butanol, ethyl acetate) are higher than in a polar solvents like chloroform. The antioxidant power is proportional to the values of proanthocyanidins found in these different solvents. Being soluble in water they are well present in hot water infusions.[19] The concentration in decoctions is often higher than in simple infusions. Molecules with a high degree of polymerization, although

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water soluble, probably dissolve in larger quantities in a decoction. In the leaf of tea (*Camellia sinensis*) the degree of polymerization of proanthocyanidins is only 1.02 and in the stem it is 3,13.[20–23] If this hypothesis is true, this becomes very important. It was always believed that it was important to make the infusion with 90°C water. But Artemisia plants are rich in proanthocyanidins. It would thus be important to use decoctions in lieu of infusions.

Prophylaxis

Natural, herbal medicine has probably been able to protect humans against tropical diseases in a prophylactic way. More recent anecdotic inputs we received from many of our partners on the prophylactic effect of Artemisisia plants justify more research on this crucial issue. Pharmaceutical companies have over the last 50 years raised tremendous hopes but failed in developing a vaccine against malaria.[24] If we were able to better understand the polytherapy of plants we might be able to grow and condition them in a more professionnal and hygienic way, present them in a galenic form accessible to poor populations in the most remote places. The WHO opens the door to this ambitious attempt by its WHO Traditional Medicine Strategy 2014–2023 statement and the Nagoya Protocol of 2014. Traditional use refers to documentary evidence that a substance has been used over three or more generations of recorded use for a specific health related or medicinal purpose (WHO/EDM/TRM/2000.1). In this case WHO maintains its position that there is no requirement for pre-clinical toxicity testing. Pre-clinical toxicity testing is only required for new medicinal herbal products which contain herbs of no traditional history of use.

Punica granatum (pomegranate) peels are very rich in proanthocyanidins 1200mg/100g.[25] Another study finds 27600mg/100g.[26] The prophylactic properties of this fruit are well known in India. Since 10 years a home-made biomedicine called OMARIA is on the market. The powder of the sundried pomegranate skin is administered in gelatin capsules in several villages. A report documents the results obtained with 401 patients of all ages.[27–29] After one year, only 5 people suffered a malaria infection. The vast majority became asymptomatic. During the same period the neighboring villages which were not treated reported and continue to report high incidence of malaria, up to 6 afflictions for the same person, including cerebral malaria. Several individual cases have been continuously followed up for years. There wasn't any indication of resistance to OMARIA. No side effects were noticed, nor antagonism with other antimalarial drugs. The Red Cross Society of the Orissa district is managing the project. Beta-hematin inhibition correlates positively with antimalarial properties A strong beta-hematin inhibition of aqueous extract of pomegranate peels was confirmed (Mutaz Akkawi, Palestine, personal communication). Other research teams study prophylaxis with the fruit rind of pomegranate in Gabon and in Italy.[30,31]

Another plant which has strong prophylactic properties against malaria is *Azadirachta indica*.[32,33] Neem is very rich in proanthocyanidins: 6500mg/100g.[34] But it has only very low if any curative and suppressive properties.[35] Cocoa (*Theobroma cacao)* was known by the Maya as diet-mediated antimalarial prophylaxis. Based on this anecdotal information prophylactic trials have been started in Ghana by the Ghana Cocoa Board. People are encouraged to daily drink a beverage made by mixing boiling-hot water and natural cocoa powder.[36] Natural cocoa powder has measurable direct *in vitro* inhibitory effect on *Plasmodium falciparum*. This supports anecdotic

reports of its ability to prevent malaria, as a result of regular beverage intake.[37] This has been confirmed by *in vivo* trials in mice. Cocoa powder was equivalent to chloroquine and has also prophylactic properties.[38] It also raises CD^{4+} T-cell counts, an effect similar as the one documented by Constant Kansango & Pierre Lutgen[39,40] for *Artemisia afra*. Cocoa powder is effective in attenuating adverse effects of ACT treatment. For example, proved cardio protective and renoprotective potential during artemether-lumefantrine administration.[41,42]

A more recent paper confirms the strong antimalarial properties of aqueous leaf extract of *Theobroma cocoa*. The effect is very strong against the W2 chloroquine resistant *Plasmodium falciparum* strain: IC50 of 0.7µg/mL versus 3.4 for *Persea americana* and 4.4 for *Tridax procumbens,* 2 other known antimalarial plants.[43] Proanthocyanidins are much higher in *Theobroma cocoa* leaves than in grains: 15% DW vs 4%.[44] The Kuna Indians, who reside in an archipelago on the Caribbean Coast of Panama, have very low blood pressure (BP) levels, live longer than other Panamanians, and have a reduced frequency of myocardial infarction, stroke, diabetes mellitus, and cancer. One outstanding feature of their diet includes a high intake of flavanol-rich cocoa, which activate nitric oxide synthesis in healthy humans. Nitric oxide (NO) is an important weapon against Plasmodium.[45] The epicatechin from *Theobroma cocoa* actively increases nitric oxide synthesis in the body.[46] Avocado seeds (*Persea americana*) are rich in proanthocyanidins.[47] Proanthocyanidins have a strong ferrous ion chelating power and NO production ability.[48] This may explain the prophylactic power of the drug Artavol[R] developed by Patrick Ogwang in Uganda. There is growing evidence that arginine, proanthocyanidins present in Artemisia plants and the nitric oxide they generate, interfere with the intrahepatic malaria parasites.[49–51]

Proanthocyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should limit their absorption through the gut barrier, unless membrane carriers are involved. They reach the small intestine almost intact, where they are hardly absorbed because of this high molecular weight. *In vitro* and *in vivo* studies using pure compounds as substrates suggest that proanthocyanidins may be degraded into more bioavailable low-molecular-weight phenolic acids by the microflora in the colon. Dimers and trimers are able to cross the intestinal epithelium. Proanthocyanidins are stable during gastric transit in human. Because of their stability they may provide a continuous supply of metabolites.[52] Saponins well present in Artemisia plants facilitate however the uptake of other materials to which the gut would normally not be permeable.[53] The fact that tannic acid forms complexes with gelatin might have an impact on the use of gelatin capsules for the administration of powdered leaves of medicinal plants. In as seminal work on simulated digestion Weathers[54] found that dried leaves in gelatin capsules reduced by >50% the amount of artemisinin released in intestinal liquid, but the amount of released flavonoids nearly doubled.

Vegetable oils used as dietary supplementation only moderately reduced the release of artemisinin but doubled the release of flavonoids. The effect of millet or corn on artemisinin release was more pronounced. We would propose as working hypothesis that the tannins present in gelatin capsules or in millet or corn (more than in rice) react with artemisinin, either binding it or acting as probiotics and metabolizing it. The increase in flavonoids noticed in this study (P Weathers op.cit.) might be related to a depolymerization of proanthocyanidins into catechin or gallic acid. Another important

factor is that tannins enhance the solubilization of hydrophobic molecules and drugs. The solubility of the anti cancer drug docetaxel for example is increased from the µg/mL level to the mg/mL level.[55] This may also explain the surprisingly high solubility of artemisinin in tea infusions. And the solubility of resveratrol in wine and not in pure water. Proanthocyanidins act like glues and are extracted as adhesives from coffee bran and pine needles. Or from agricultural wastes to produce biological glues in lieu of chemical glues.

Powdered leaves of Artemisia easily produce a sticky mass when wetted, probably because they are rich in condensed tannins.[56,57] They are used as a protein fixative in electron microscopy. And as it is well known that malaria parasite proteins remodel the host erythrocyte this may be an important factor in antiplasmodial properties.[58] Hemoglobin acts as binding substrate in the quantitative analysis of plant tannins or in dentistry.[59–61] They bind precipitate and form complexes with proteins.[62] This is one of the reasons why they are used in wine fining to remove turbidity. They form insoluble, nonputrescible precipitates with albumin, gelatin, and proteins. It is known since 100 years that gelatin is crosslinked by proanthocyanidin.[63] This crosslinking effect by proanthocyanidins may affect the specific proteins generated by *Plasmodium falciparum* to induce changes in the morphology, physiology and function in the erythrocyte host cell. Sporozoites are the infective stage of Plasmodium for the human host. It has been demonstrated that circumsporozoite protein CSP and the transmembranal protein TRAP are essential, insuring the gliding motility for the sporozoite to reach the liver. These proteins uniformly cover the external surface of the sporozoite.[64]

The fact that proanthocyanidins form complexes with proteins may strongly affect the gliding motility of sporozoites. Electron microscopy of sporozoites of the rodent malaria parasite, *Plasmodium berghei*, reveals electron-dense multilaminate membranous whorls after fixation with tannic acid.[65,66] They change the 'stick-and-slip' parasite motility supported by TRAP.[67] It is known that reducing the protein content of the diet leads to a decrease in the level of infection. Undernutrition may even protect against severe malarial infection. Binding with proteins proanthocyanidins might thus have a positve effect on malaria.[68] CPAC Condensed tannins of cranberry and pomegranate reduce the motility of *Pseudomonas aeruginosa*. The effect of proanthocyanidins on the motility of fish spermatozoides has been demonstrated: a reduction proportional to tannin concentration.[69] Bacterial motility which plays a key role in the colonization of surfaces by bacteria is also blocked by proanthocyanidins[70] but it depends on the type: extracts from cranberry are effective, extracts from cinnamon not. Condensed tannins have the ability to reduce the population of protozoa in the rumen of ruminant species.[71] Some hundred years ago it was found that tannic acid kills Paramecium and other protozoans in waste water.[72]

Several organic acids were used over the same pH range. Acetic, citric and formic acids elicited responses somewhat comparable to those of the inorganic acids. Pyrogallic and tannic acids proved to have such a high degree of toxicity for Paramecia that reactions within the pH range studied were impossible, death resulting on contact with the weakest part of the diffusion area. This high degree of toxicity of tannic acid may offer an explanation of the absence of Protozoa from bog (wetland) waters. This is in line with some recent findings at the University Paris Sud. *Artemisia annua* kills *Paramecium tetraurelia*. The effect of *Artemisia afra* is even stronger. A recent study reveals that the human gut bacteria *Escherichia coli*, normally considered

a microbe of pathogenic potential within the host, may help us fend off malaria infection.[73] Host organisms recognize pathogen-associated molecular patterns, including glycans. During evolution, selective pressures for an effective human immune system result in genetic polymorphisms that prevent the biosynthesis of certain glycans, including Galα1-3Galβ1-4GlnAc-R (α-gal) in humans. Thus, human exposure to common α-gal-positive pathogens, including enterobacteria, such as *Escherichia coli*, induces the production of high titers of circulating antibodies against α-gal. A team from Portugal found that *Plasmodium falciparum* expresses the same glycan molecule as *E.coli*. It is thus logical to expect that antibodies to α-gal cytotoxic to *E.coli* are also cytotoxic to Plasmodium. Anti-α-gal antibodies bind to the sporozoite surface, induce their lysis and prevent Plasmodium invasion of hepatic cells. Condensed tannins chelate iron and zinc.[74,75]

Plasmodium falciparum badly needs these metals for its growth and replication. The role of iron is well known, the role of zinc less. The parasite-driven fluxes of weakly bound zinc into infected erythrocytes are essential to pathogenicity.[76] The *Plasmodium falciparum* parasite requires acquisition of 30 million zinc atoms before host cell rupture, corresponding to a 400% increase in total zinc concentration. Highly zinc-specific chelators are shown to inhibit the growth of the parasite while causing an arrest in development at the trophozoite stage. If enough chelator is available survival of the parasite is no longer possible. These chelator treatments result in a depolarization of Plasmodium mitochondria, which suggests a mechanism of chelator-induced cell death via mitochondrial disruption. But the action of these chelators is slow, up to 48 hours but stable. Proanthocyanidins are prophylactic, but they are also curative. This is the case for many spices and plants, especially the bark or stems of these.[77]

Proanthocyanidins are characterized by a great stability in soil and mammal metabolism. Tannins may inhibit enzyme activity and thus exert a negative effect on microbial growth. A USDA work showed that non-tannin phenolics and hydrolizable tannins are rapidly metabolized. Condensed tannins as for example sorghum tannin are very stable.[78–80] Condensed tannins are hard to be degraded in both aerobic and anaerobic environnements.[81] This stability explains their longlasting allelopathy; in the digestive tract and in the conservation of food.[82] They act more slowly than artemisinin. In a comparative assay on the effect of artesunic acid versus *Carica papaya* leaf extract the former had strong effect after 4 days and the latter only after 7 days.[83] It may be the key for the prophylactic effect evident after regular Artemisia tea consumption, even in minor quantities. A permanent reservoir of condensed tannins in the human body.

Acknowledgements

Conflict of interest

The author declares that there is no Conflict of interest

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