Tannins in Artemisia: hidden treasure for prophylaxis

October 9, 2016

https://malariaworld.org/blog/tannins-artemisia-hidden-treasure-prophylaxis

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.

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 proanthocyanidins 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 (Liwei Gu et al., Am Soc Nutritional Requirements 2003 Dec, 613-617). 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 (1 900), 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 (8 100). 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 (B Labarbe et al., J Agric Food Chem. 1999, 47, 2719-23). In sorgho the degree of polymerization is higher than 10. In cranberries dimers and trimers predominate.

It is very difficult to find studies on proanthocyanidins in Artemisia plants. In Artemisia afra very high concentrations were found: 19 900 mg/100g (TO Sunmonu Pak J Nutrit 2012, 11, 520-525). A very recent paper deals with Artemisia herba alba and finds a concentration of 2 100 mg/100g (LS Eddine et al., J Pharmacy Res, 2016, 10, 58-64). 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 (M Mojarab et al., Pharmacologonline 2009, 2, 797-807). A more recent paper from Iran finds 340 mg/ 100 g in Artemisia annua leaves and only 30 mg/100g in stems (M Mazandarani et al., J Med Plants and By-products, 2012. 1, 13-21). A paper from Turkey finds an average of 420 mg/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 600 mg/100g DW, for Fabiaceae 400 , for Artemisia campestris 5 700 and for A.herba alba 3 600 mg/100 g (S Boufennara et al., Span J Agricult Res. 201210, 88-98). Tannin in A absinthium varies significantly from one region in Tunesia to another, from 400 to 1200 mg/100g (K Msaada et al., Journal of Chemistry Volume 2015, Article ID 804658). Artemisia plants are very rich in tannins (Rosine Chougouo, personal communication, 2016). The big spread in analytical data of course is also due to different analytical methods. A standardized technique needs to be defined and implemented.

A fascinating property of Artemisia infusions is that they protect mild steel against corrosion. It was suggested that artemisinin might be responsible for this property, but it is not very logical as artemisinin is a peroxide (F Okafor et al., Int. J. Electrochem. Sci., 7 (2012) 11941 – 11956). Furthermore also other Artemisia species not containing artemisinin show a similar effect. Comparing the anticorrosion properties of some 10 plants an Arabian study found that A sieberi ranks at the top (M.S. Al-Otaibia, Arabian Journal of Chemistry,July 2014, 340–346). Other plants rich in tannins also inhibit steel corrosion like Azadirachta indica (Okafo et al ., Int. J. Electrochem. Sci., 2010, 5, 978 – 993). Neem bark contains 14% tannin, an amount similar to that in conventional tannin-yielding trees. Or Punica granatum (H Ashassi-Sorkhabi et al., Int. J. Electrochem. Sci., 2010, 5, 978 – 993) or grape seed pomace. (JC da Rocha, International Journal of Corrosion, 2015, Article ID 197587). One of the reaction mechanisms of tannins with rust apparently involves three general steps: (i) adsorption of the tannin to the rusty surface, (ii) complexation of ferrous/ferric ions followed by dissolution, and (iii)partial or complete readsorption of the iron-tannate complexes to the substrate. A blue-black coating layer is formed.

Artemisia plants often show allelopathic properties like other plants which have a high content in proanthocyanidins (see the blog Artemisia, allelopathy, tannins on www.malariworld.org).

Proanthocyanidins over recent years have been extensively extracted from agricultural wastes to produce biological glues in lieu of chemical glues. Powdered leaves of Artemisia leaves easily produce a sticky mass when wetted, probably because they are rich in condensed tannins.

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 6 800 to 13 000mg/100g DW. Plants like Moringa oleifera or Panax ginseng who do not contain condensed tannins have no antimalarial properties.

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.

ANTIOXIDANT PROPERTIES OF TANNINS

The antioxidant properties of plants are to a large extent related to their content in proanthocyanidins. Their antioxidant capacities are higher or comparable to that of vitamin C or E (D Rösch et al., Eur Food Res Technol 2004 219:605). 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 (G Plumb et al., Free Radical Res., 1998 29:4). The plant with the highest total antioxidant capacity (TAC) are Sorghum sumac leaves. In the same study JF Ferreira found that Artemisia afra also has a high TAC, higher than Artemisia annua (Brisibe, E.A et al., Food Chem., 115: 1240-1246.2009). 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 (I Carvalho et al., Industrial Crops and Products, 2011, 33, 382-388).

Concentrations found in polar solvents (water, butanol, ethyl acetate) are higher than in apolar solvents like chloroform. The antioxidant power is proportional to the values of proanthocyanidins found in these different solvents (LS Eddine et al., J Pharmacy Res. 2016, 10, 58-64). Being soluble in water they are well present in hot water infusions.

The concentration in decoctions is often higher than in simple infusions. Molecules with a high degree of polymerization, although water soluble, probably dissolve in larger quantities in a decoction. In the leaf of tea (Camellia sinensis) the degree of polymarization of proanthocyanidins is only 1.02 and in the stem it is 3,13..

Xiaolan Jiang, Yajun Liu, Yahui Wu. Analysis of accumulation patterns and preliminary study on the condensation mechanism of proanthocyanidins in the tea plant [Camellia sinensis]. Sci Rep. 2015; 5: 8742. doi: 10.1038/srep08742

Hanadi A. Attieh, Saleh Abu Lafi, Suhair Jaber, Qassem Abu-Remeleh, Pierre Lutgen and Mutaz Akkawi. University, West Bank, Palestine 3 IFBV-BELHERB, Luxembourg. Cinnamon bark water-infusion as an in-vitro inhibitor of β-hematin formation. Journal Medicinal Plants. Accepted 5 October, 2015

Su-Chen Ho, Pei-Wen Chang. Inhibitory effects of several spices on inflammation. Am J Plant Sc, 2012, 3, 995-1002

N Martins, L Barros, I Ferreira, Decoction, infusion and hydroalcoholic extract of Origanum vulgare. Food Chemistry, 2014, 158, 73-80.

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

PROPHYLACTIC PROPERTIES OF TANNINS

Natural, herbal medicine has evidently 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. 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. Both confirm previous statements: 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 1 200mg/100g (Zam Wissam et al., Intern J Pharmac Pharmaceut sc. 2012 4 ISSN-0975-1491). Another study finds 27 600 mg/100g (H Mohammed, Mesopotamia J of Agricol 2008, 36,-1). 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 sun dried pomegranate skin is administered in gelatin capsules in several villages. A report documents the results obtained with 401 patients of all ages (D Bhattacharya, Asian J Trop Disease, 2011,. 142-149 and Brit J Pharmac Res. 2012,3, 54-77). 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 manageing 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 (J Lekana-Douki et al., Inter J Clinical Med 2012, 3. 1-8) and in Italy (M Dell’Agli et al., Malaria Journal, 2010 9:208).

Another plant which has strong prophylactic properties against malaria is Azadirachta indica (P Nagendrappa et al., J Ethnopharmacol. 2013. 146, 768-772). Taken continuously during several months they seem to act at homeopathic levels (NM Barlow-Benschop, Homeopathy, Nov 15, 2006). Neem is very rich in proanthocyanidins: 6 500mg/100g (B Pokhrel et al., Afr J Biotechnology 2015, 14, 3159-3163). But it has only very low if any curative and suppressive properties (J Momoh et al., Int J Current Res., 2015, 7, 13769-13778).

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 (FK Addai, Medical Hypotheses 210,10, 825-830).

Avocado seeds (Persea americana) are rich in in proanthocyanidins (WM Chai et al., J Agric Food Chem 2015, 63, 7381-7). This may explain the prophylactic power of the drug Artavol[R] developed by Dr Patrick Ogwang in Uganda.

POSSIBLE MECHANISMS FOR THE PROPHYLACTIVE ACTION

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 (L Rios et al., Am J Clin Nutr 2002, 76, 1106-10). 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 P Weathers found that dried leaves in gelatin capsules reduced by >50% the the amount of artemisinin released in intestinal liquid, but the amount of released flavonoids nearly doubled (PJ Weathers et al., J Ethnopharmacol 2014, 151, 858-863). 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 anticancer drug docetaxel for example is increased from the µg/mL level to the mg/mL level (JK Jackson et al., J Pharm Sci 2016 xphs 2016.06.027). 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.

Protoanthocyanidins act like glues and are extracted as adhesives from coffee bran and pine needles (X Zhou et al., Int Wood Prod J. 2014. 527-32). They are used as a protein fixative in electron microscopy (KM Meek et al., Biochim Biophys Acta 1979, 587, 112-20). Hemoglobin acts as a binding substrate in the quantitative analysis of plant tannins (Jack C. Schultz, et al.,J. Agric. Food Chem., 1981, 29 (4), pp 823–826). They bind and precipitate and form complexes with proteins (J

Van Buren et al., J Agric Food Chem 1969 17, 772-774). 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 (AW Thomas et al., Industrial and Egineering Chemistry, 1923, 15, 839-841)) and (S Kim et al., J Biomed Mater Res B Appl Biomat 2005, 75 442-50). 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. Cranberry proanthocyanidins attach Bacillus cereus bacteria to substrates (AR Jones, Thesis Georgia State University, 2007).

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 (A Sultan et al., Cell Vol. 1997, 90, 511-522). 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. (Stewart MJ et al., J Protozool. 1985 May; 32:280-3). 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 (DL Waltemeyer, Trans Amer Fisheries Soc 1975, 104-4). Bacterial motility which plays a key role in the colonization of surfaces by bacteria is also blocked by proanthocyanidins (Che O'May et al., Appl and Envir Microbiol. 2011,77, 3061-3067) 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 (A Pineiro-Vasquez et al., Archivos de Medicina veterinaria 2015 47, ISSN 0301-732X). Some hundred years ago it was found that tannic acid kills Paramecium and other protozoans in waste water (WH Johnson, Biological Bulletin, 1929, LVII,199-224). 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 (Jérôme Munyangi, personal communication, 2015). Artemisia annua kills Paramecium tetraurelia. The effect is even stronger for Artemisia afra.

Condensed tannins chelate iron (AT Iffat et al., Jour Chem Soc Pak 2005 27, 174) and zinc (JD Hem, (M Karamac, Int J Mol Sc 2009,10, 5485-5497). 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 (RG Marvin et al., Chemistry & Biology 2012, 19, 731-741). The Plasmodium falciparum parasite requires acquisition of 30 million zinc atoms before host cell rupture, corresponding to a 400% increase in total zinc concentration. The same authors showed that the zinc chelators TPEN (tetrakis-pyridylmethyl-ethylene-diamine), EDTA (ethylenediaminetetraacetic acid), TETA (triethylene-tetramine), DMSA (dimercaptosuccinic acid) were highly active against the Plasmodium parasite at micromolar concentrations. 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 characterized by a great stability in soil (B Smolander at al., Soil Biology and Biochemistry, 2011,43, 628-637), a property which explains their long lasting allelopathy ; in the digestive tract (L Rios op.cit.) and in the conservation of food (G Bartosz, Food antioxidants, CRC Press 2014). This 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.

Pierre Lutgen