Saponins, tuberculosis and malaria

A team of medical doctors in Nord Kivu discovered that *Artemisia afra* infusions were very efficient in the therapy of tuberculosis patients.

Daddy B, Lutgen P, Gisenya P. Breakthrough against tuberculosis: high efficacy of Artemisia afra infusions. Pharm Pharmacol Int J. 2021;9(2):58–62. DOI: 10.15406/ppij.2021.09.00328

Some other plants are also known to have an antituberculosis effect. For example, *Entada abyssinica,* a Fabaceae, rich in saponins was found to be be very efficient *in vitro* against *Mycobacterium tuberculosis*.

Richard M. Mariita, John A. Orodho, Paul O. Okemo, Paul K. Mbugua. Antifungal, antibacterial and antimycobacterial activity of Entada abysinnica Steudel ex A. Rich (Fabaceae) methanol extract, Journal: Pharmacognosy Research, : 2010, ISSN: 0974-8490

The pharmacological activity of *Centella asiatica* is attributed to saponins i.e. centellosides, mainly asiaticoside The plant contains up to 4% of saponins on dry weight and is used in China against leprosy. This is known since 1949.

P Boiteau, A Buzas, Derivatives of Centella asiatica used against leprosy. Nature Vol 163, 1949.

Centella asiatica is also active against Mycobacterium macrophages.

A Mustika, N M Mertaniasih. Ethanol extract of Centella asiatica reduces expression of Mycobacterium tuberculosis alveolar macrophages from rats lung tissue. Pathology, 2016, 48(81), 144.

Suresh M., Rath P.K., Panneerselvam A. Anti-Mycobacterial Effect of Leaf Extract of Centella asiatica . (Mackinlayaceae). Research J. Pharm. and Tech. 3(3): July-Sept. 2010

Saponins (Latin "sapon"), also referred to as triterpene glycosides, are bitter-tasting plant-derived organic chemicals that have a foamy quality when agitated in water.

Saponins are widely distributed among flowering plants and serve in defense for these organisms. Their amphiphilic molecules are composed of a lipophilic aglycone and one or more hydrophilic sugar moieties, giving them a high

degree of structural diversity and their useful soap properties. Their biological and pharmacological activities range from antimicrobial, antifungal, to immunomodulatory.

They are also used in soaps, fire extinguishers, and in carbonated beverages (the head on a mug of beer).

The amphiphilic nature of saponins gives them activity as surfactants with potential ability to interact with cell membrane components, such as cholesterol and phospholipids, possibly making saponins useful for development of drugs.

Membrane permeability effects

The most prominent feature of saponins is linked to their effects on cell membranes; they strongly affect cell membrane structure and integrity by different mechanisms depending on their chemical structure. The ability of saponins to increase membrane permeability can be used to facilitate the passage of drug molecules or other natural products through the cell membrane. The ability of saponins to affect cell membrane structure and integrity makes them interesting natural products in pharmacological and medical research and therapy, in particular as agents for enhancing drug efficacy.

Sudji, Ikhwan Resmala. Bioactivity of Steroid and Triterpenoid Saponins: Influence on Membrane Permeability and Drug Absorption. Dissertation. : http://www.ub.uni-heidelberg.de/archiv/19012

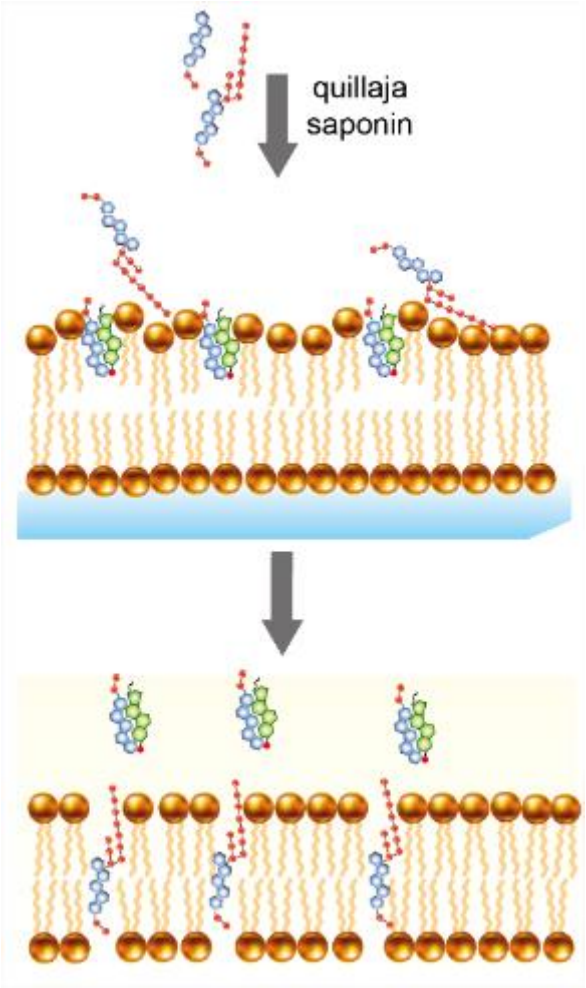
De Geyter, E., Swevers, L., Caccia, S., Geelen, D. and Smagghe, G. (2012), Saponins show high entomotoxicity by cell membrane permeation in Lepidoptera. Pest. Manag. Sci., 68: 1199-1205. https://doi.org/10.1002/ps.3284

To investigate the molecular activity of substances on the membrane system, it is necessary to understand the critical aspects of membrane structure and its properties. Biological membranes are dynamic structures composed of a diverse set of lipid components such as phospholipids, glycolipids, sterols, and proteins. The fundamental structural component of all biological membranes is the lipid bilayer which is made up of two opposing layers of lipid molecules, where the hydrophilic polar head is directed outwards towards the aqueous

phase and lipophilic tails pointing at the interior of the two layers. The hydrophobic interior of lipid bilayers acts as a barrier to limit the movement of polar molecules and ions in or out from the cell. The membrane's structural integrity protects the cell against various adverse external factors, in particular against toxic chemical compounds and xenobiotics.

Formation of membrane pores by saponins is not specific or restricted to particular kinds of cells. The pore-forming effect of saponins is often used to render membranes more accessible to polar substances for enhancing drug efficacy.

Quillaja saponin, for example, affects bilayer membranes, increases membrane permeability, but leaves the membrane intact.



A German research paper shows that the incubation of cells and tissues with saponin makes the lipid bilayer permeable to macromolecules, by affecting the interaction between transmembrane proteins and the cytoskeleton.

Baumann E, Stoya G, Völkner A, Hemolysis of human erythrocytes with saponin affects the membrane structure. Acta Histochem. 2000 Feb;102(1):21-35

Studies on cancer cells have shown that saponins can induce apoptosis through a series of reactions involving the activation of a protease family of enzymes known as caspase. *Mycobacterium tuberculosis* hides in macrophages and granuloma cells. When this shelter ruptures in necrosis, it releases the Mycobacteria which generate new infections. Necrosis, a form of cell injury, due for example to bacterial toxins, results in the premature death of cells which often ruptures. This does not apply to apoptosis. Apoptosis is a programmed form of cell death characterized by cell shrinkage and membrane blebbing. Apoptosis is well regulated and not accompanied by an inflammatory response.

Some saponins also inhibit CYP3A4 and this may enhance drug bioavailabilty.

Wang M, Jiang W, Zhou J, Xue X, Yin C. Anemarsaponin BII inhibits the activity of CYP3A4, 2D6, and 2E1 with human liver microsomes. Pharm Biol. 2020 Dec;58(1):1064-1069. doi: 10.1080/13880209.2020.1835996..

Several of our partners found that *Artemisia annua* is a strong inhibitor of CYP3A4, even stronger than ketaconazole or grapefruit juice.

LAZARIDI Kristina. Invloed van de chemische samenstelling van Artemisia annua op CYP3A4-activiteit en antioxidant vermogen. Thesis. VUB. 2013-14

Desrosiers MR, Mittelman A, Weathers PJ. Dried Leaf Artemisia Annua Improves Bioavailability of Artemisinin via Cytochrome P450 Inhibition and Enhances Artemisinin Efficacy Downstream. Biomolecules. 2020 Feb 7;10(2):254. doi: 10.3390/biom10020254. PMID: 32046156; PMCID: PMC7072484.

The effect of the CYP3A4 inhibitor ketoconazole was studied on *Mycobacteria tuberculosis in vitro* and *in vivo* in a murine model. The *in vivo* activity of KTC was evaluated in established pulmonary TB in the murine model, alone and in combination with isoniazid, pyrazinamide, and rifampicin. KTC alone exhibited little effect after short-term treatment. KTC, when added in combination with

these drugs significantly improved the treatment outcome in the lungs compared with the drugs alone. Further investigation is necessary to determine the role of KTC in the treatment of TB. A study found that it had an effect on the respiratory distress syndrome. A fascinating new field of research !

Byrne ST, Denkin SM, Gu P, Nuermberger E, Zhang Y. Activity of ketoconazole against Mycobacterium tuberculosis in vitro and in the mouse model. J Med Microbiol. 2007 Aug;56(Pt 8):1047-1051. doi: 10.1099/jmm.0.47058-0. PMID: 17644711.

John G. Williams, Ronald V. Maier Ketoconazole inhibits alveolar macrophage production of inflammatory mediators involved in acute lung injury (adult respiratory distress syndrome). Society of University Surgeons, 122-2,270-277. doi.org/10.5555/uri:pii:003960609290220T

Immunomodulatory effects

The anti-inflammatory effect of the saponin extracts of 5 Nigerian plants rich in saponin : *Schwenkia americana* (2.74% saponin), *Asparagus africanus* (3.59%), *Dichrostachys cinerea* (1.62%), *Ficus iteophylla* (0,81%), and *Indigofera pulchra* (1.57%) has been described and is equivalent to Ketoprofen.

H S Hassan, M I Sule,. Anti-Inflammatory Activity of Crude Saponin Extracts from Five Nigerian Medicinal Plants. Afr J Tradit Complement Altern Med. 2012; 9(2): 250–255.

Saponins have also been used as adjuvants in development of vaccines, such as an extract from the bark of *Quillaja saponaria*. This makes them of interest for possible use in subunit vaccines and vaccines directed against intracellular pathogens.

Saponin based adjuvants also have the ability to stimulate the cell mediated immune system as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity. Adjuvant functions include stimulation of high levels of antibody to T-dependent and T-independent antigens, induction of mouse IgG1, IgG2b, and IgG2a isotypes, and induction of cytotoxic T lymphocyte responses.

Rajput ZI, Hu SH, Xiao CW, Arijo AG. Adjuvant effects of saponins on animal immune responses. J Zhejiang Univ Sci B. 2007 Mar;8(3):153-61.

C R Kensil . Saponins from Quillaja saponaria as vaccine adjuvants. Crit Rev Ther Drug Carrier Syst. 1996;13(1-2):1-55. PMID: 8853958

Chioma Miracle Ojiako, Ebere Innocent Okoye, Preliminary studies on the formulation of immune stimulating complexes using saponin from Carica papaya leaves. Heliyon, Vol 5, Issue 6, 2019, doi.org/10.1016/j.heliyon.2019.

Saponin as coadjuvant was very efficient in a vaccine against hemorrhagic septicemia in mice and buffalo calves, increasing CD4 and CD8 cell populations and improving the humoral immune response. Similar effects have been described by M-A Lacaille-Dubois (Immunomodulatory Agents from Plants, ed Paniham, 1999).

Administration of a medium dose of *Dioscorea nipponica* saponin was found to dramatically increase the percentage of CD4(+) cells in bone marrow nucleated cells

Wang Y, Yan T, Ma L, Liu B. Effects of the total saponins from Dioscorea nipponica on immunoregulation in aplastic anemia mice. Am J Chin Med. 2015;43(2):289-303. doi: 10.1142/S0192415X15500196. Epub 2015 Mar 19. PMID: 25787297.

The adjuvant has a long range effect and IgGs continuously increase during weeks after inoculation.

de Costa F, Yendo ACA, Cibulski SP, Fleck JD, Roehe PM, Spilki FR, et al. (2014) Alternative Inactivated Poliovirus Vaccines Adjuvanted with Quillaja brasiliensis or Quil-A Saponins Are Equally Effective in Inducing Specific Immune Responses. PLoS ONE 9(8): e105374. doi.org/10.1371/journal.pone.0105374

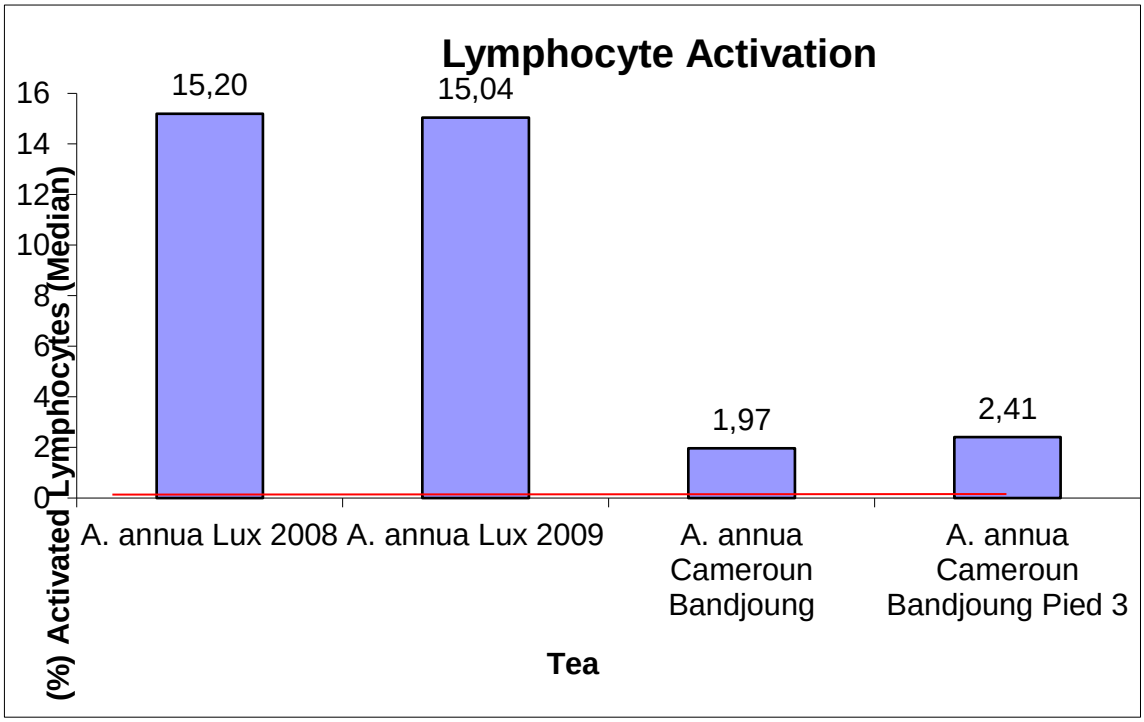
Saponin is also used as an adjuvant in antimalarial vaccines. Malaria vaccine (with Quillaja saponin as component of the adjuvant) is currently under review for the regulatory application to European Medicines Agency to be licensed for human use

McColm AA, Bomford R, Dalton L. A comparison of saponin with other adjuvants for the potentiation of protective immunity by a killed Plasmodium yoelii vaccine in the mouse. Parasite Immunol. 1982 Sep;4(5):337-47. doi: 10.1111/j.1365-3024.1982.tb00445.x. PMID: 7145465.

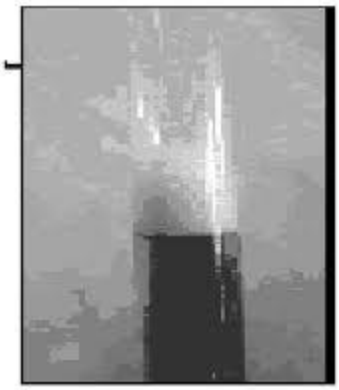
Saponins are also used in curare poisons. *Abuta grandifolia* which is used through South America as an antimalarial drug is also traditionally used as an ingredient in curare arrow-poisons. Their high saponin content could favourize alkaloid solubilisation and cell permeation.

C Lavaud, C Sayagh , C Long , C Moretti.Alkaloids and saponins from twigs and leaves of Abuta grandifolia (Mart.) Sandw Planta Med 2008; 74 - PB112. DOI: 10.1055/s-0028-1084457

In 2010 we noticed in a laboratory in Luxembourg (personal communication) a much stronger activation of lymphocytes by *Artemisia annua* tea from Luxembourg than *Artemisia annua* from Cameroon.



We tried to find an explanation, but the only difference we found was that the tea from Luxembourg contains much more saponins as per the foam or froth test



As it is difficult to perform a quantitative analysis of the saponin content in a plant, due to the complexity of the molecular composition and the spectra obtained, the qualitative frothing test is often used to determine the presence of saponins. A few mL of the aqueous extract are shaken vigorously for 4 minutes and the presence of honey-comb froth indicates the existence of saponins. The froth needs however to stay stable for a few minutes

Saponin content in different plant parts

Based on this intriguing finding on differences in lymphocyte activation and saponin content we tried to better understand the presence of saponins in different plant parts and their evolution with the age of the plant. Saponins are found in plants because they have an insecticidal role.

Cui C, Yang Y, Zhao T, et al. Insecticidal Activity and Insecticidal Mechanism of Total Saponins from Camellia oleifera. Molecules. 2019;24(24):4518. Published 2019 Dec 10. doi:10.3390/molecules24244518

In *Saponaria officinalis* the greatest concentration of saponin will be when the plant is flowering, and the most saponin is found in the woody parts of the plant like thick stems and roots, but the leaves also have some.

In eight plant species found in Quetta it was noted that the level of saponin were comparatively found greater in stem over their respective leaves.

Abdul Kabir Khan Achakza, Palwasha Achakza, Response of plant parts and age on plants found in Quetta.Pak. J. Bot., 41(5): 2129-2135, 2009.

In the plant *Mimosa pudica* saponin concentration in aqueous extracts is higher in stems than in leaves (1.55% versus 1.30%). All other constituents like alkaloids, flavonoids, tannins, glycosides are lower in stems.

I Ibrahim. Qualitative and Quantitative Phytochemical Screening of Mimoso Pudica Plant Extracts (Touch Me Not) Am J Biol Chem 2014, 2, 8-16).

In *Carica pubescen*s higher saponin content is found in petiole (twigs) than in leaves

Minarno, Eko Budi; LAILY, Ainun Nikmati; Alfiah, Ida. Saponin Content Analysis on Leaves and Petioles of Carica pubescens Lenne & K. Koch. Proceedings of the International Conference on Green Technology, [S.l.], v. 8, n. 1, p. 311-318, nov. 2017. ISSN 2580-7099.

The observation that saponin content is higher in stems than in leaves may explain why *Artemisia annua* from Cameroon does not activate lymphocytes. The sample received from the Université des Montagnes only contained powdered leaves without stems.

The most complete study for the plant *Achyrantus bidentata* on accumulation and dynamics of saponins in vegetative organs has been run in China over the months from August to November. In the leaves the saponin decreases from 4.09% in August to 0.06% in November, but in the stems it only decreases from 4.13 to 1.47. This may explain to some extent why leaves of *Artemisia annua* harvested in October have lost a lot of their efficiency, for example in the beta-hematin inhibition test.

Jinting Li, Zhenghai Hu. Accumulation and Dynamic Trends of Triterpenoid Saponin in Vegetative Organs of Achyranthus bidentate. J Integrative Plant Biology 2008

Some studies have found interesting relationship between the host plant and secondary metabolite contents, which indicate that attraction or resistance to herbivores is due to higher concentrations of saponins in younger leaves in contrast to the older leaves of the Barbarea genus.

Hussain M, Debnath B, Qasim M, et al. Role of Saponins in Plant Defense Against Specialist Herbivores. Molecules. 2019;24(11):2067. Published 2019 May 30. doi:10.3390/molecules24112067

Badenes-Perez FR, Gershenzon J, Heckel DG (2014). Insect Attraction versus Plant Defense: Young Leaves High in Glucosinolates Stimulate

Oviposition by a Specialist Herbivore despite Poor Larval Survival due to High Saponin Content. PLoS ONE 9(4): e95766. doi.org/10.1371/journal.pone. 0095766

It was observed that young plants have higher saponin contents than mature or old plants, although several factors such as physiological state and environmental factors affect the saponin contents.

Moghimipour, Eskandar & Handali, Somayeh. (2015). Saponin: Properties, Methods of Evaluation and Applications. Annual Research & Review in Biology. 5. 207-220. 10.9734/ARRB/2015/11674.

Another study found that young leaves and stems of *Chenopodium vulvaria* and *Oxalis debilis* contained saponin, while old leaves and stems did not.

Aisha Hassan, Palwasha Achakzai and Hasnain Nangyal. Detection and Estimation of Alkaloids, Saponins and Tannins in Herbs of Quetta Baluchistan. American-Eurasian J. Agric. & Environ. Sci., 15 (6): 985-990, 2015

A study from Yunnan showed that the content of saponin in fresh *Panax stipuleanatus* was significantly lower than that in dried products. Even if this does not address the question if dried Artemisia plants are poorer in saponins than fresh plants, it might give some useful indication. In fact, in the past before the availability of commercial soap, people used the dried roots of *Saponaria officinalis*, a common perennial plant also called soapwort, soapweed.

Shu P-P, Li L-X, He Q-M, Pan J, Li X-L, Zhu M, Yang Y, Qu Y, Identification and quantification of oleanane triterpenoid saponins and potential analgesic and anti-inflammatory activities from the roots and rhizomes of Panax stipuleanatus, J. of Ginseng Research, doi.org/10.1016/j.jgr.2020.05.002.

Saponins are bitter. Since Greco-Roman times bitter tastants have been used in Europe for various diseases. In Africa the consumption of bitter medicinal plants is higher in areas with endemic parasitic infections.

J Mennella, A Spector. The bitter taste of medicines. Clin Ther 2013 35, 1225-124

In a thesis at the University of Johannesburg the bitterness values of 15 South African plants were evaluated. The bitterness of *Artemisia afra* was 2-5 times higher than for the other plants. *Artemisia afra* was assigned a mean bitterness value of 26393. This is in line with values found by a German team of 7200-

84000 for *Artemisia afra* which is much higher than for *Artemisia absinthium* 10000-25000.

DK Olivier The ethnobotany and chemistry of South African traditional tonic plants. PhD thesis Johannesburg, 2012.

Wagner, H. & Wiesenauer, M. (2003) Phytotherapie: Phytopharmaka und pflanzliche Homöopathika; mit 103 Tabellen. Wiss. Verlag-Ges

Pierre Lutgen. Why are antimalarials bitter? www.malariaworld.org. February 7, 2020

It is also interesting to note that aqueous extracts of dry leaves contain much more saponins than alcoholic extracts.

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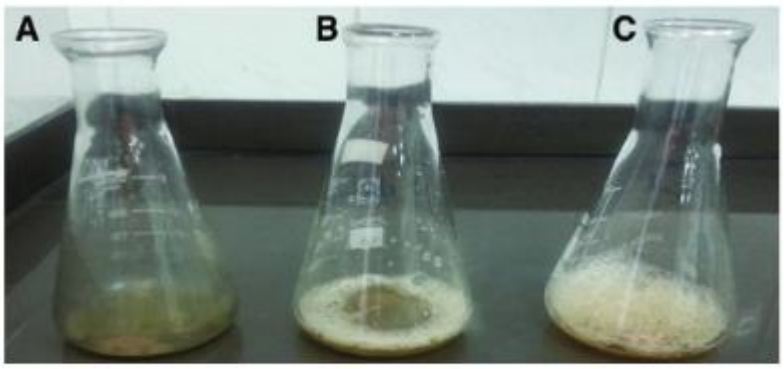


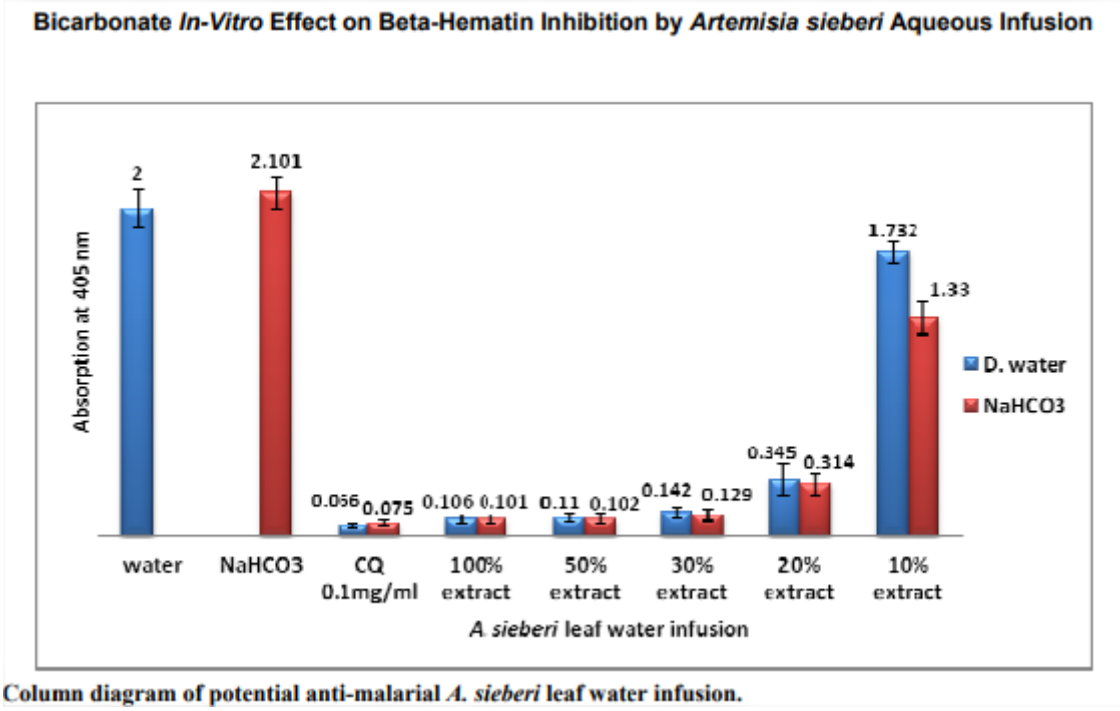
Figure 1: Generated foam by shaking the saponin extract from the *Ziziphus spina-christi* leaves using (A) ethanol, (B) methanol (C) and water.

Mahdiyeh Mohaddes-Kamranshahi, Mohammad Simjoo and Abbas Jafarizad. Evaluation of the saponin extraction from Ziziphus spina-christi leaves Green Process Synth 2019; 8: 62–67 https://doi.org/10.1515/gps-2017-0185

Quillaja saponin is soluble in water and is insoluble in most organic solvents. A 50-100 mg/ml solution in deionized water may yield a hazy solution by sonication, increased by additions of small amounts of alkali.

Missing this essential constituent of Artemisia plants, most academic studies based on extracts obtained with ethanol, hexane or other organic solvents are probably meaningless. Or in other words aqueous infusions or decoctions as we use them in the treatment of tuberculosis are the preferred approach. The fact

that the addition of alkali, i.e. sodium bicarbonate enhances many properties of Artemisia infusions was also discovered and described in several papers by Mutaz Akkawi at Al Quds University, Palestine



Column diagram of potential anti-malarial *A. sieberi* leaf water infusion.

Suhair Jaber, Saleh Abu-Lafi, Pierre Lutgen, Mutaz Qutob, Qassem Abu-Remeleh and Mutaz Akkawi. Bicarbonate In-Vitro Effect on Beta-Hematin Inhibition by Artemisia sieberi Aqueous Infusion. Journal of Pharmacy and Pharmacology 3 (2015) 63-72 doi: 10.17265/2328-2150/2015.02.003

Why the efficacy of *Artemisia afra* against tuberculosis is stronger than for *Artemisia annua* remains an open question. The peroxide artemisinin and the antioxidant saponin might have antagonistic effects. Artemisinin may cause cleavage of the saponin molecule and modify its functionality.

Anyway, saponins are not stable in aqueous solutions. Hydrolysis in lake waters (pH 6.4–8.2) produces different patterns with a fast initial dissipation of 25 to 60% of the added saponin within the first five hours, followed by an extremely slow reaction with 25 to 75% unreacted saponin left after reaction times longer than 120 h.

Jiang, Xiaogang, Strobel, Bjarne W.Stability of saponins: hydrolysis in aqueous solutions and lake waters. Environmental Science: Processes & 21, 7, doi.org/10.1039/C9EM00012G

This observation justifies our claim to drink the infusion prepared with Artemisia within the 8 first hours ; not to prepare infusions for several days ahead.

Conclusion

Mycobacterium tuberculosis hides in macrophages and granuloma cells. When this shelter ruptures, it releases the Mycobacteria which generate new infections. If saponins increase the permeability of macrophages and granuloma, some molecules present in Artemisia infusions will have an easier access to the hidden Mycobacteria and destroy them. This might explain why after a few weeks of *Artemisia afra* infusions the tuberculosis patients are completely healed without relapses.

And a few practical recommendations : use infusions and decoctions (adding eventually some alkali), don't use organic solvents or deionized water, use only fresh infusions, use leaves and petioles, twigs and stems, don't use wilted, senescent Artemisia plants, but use dried plant material and not green leaves.