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Artemisia afra infusions clinical benefits as a complementary treatment to "WHO" approved drugs against tuberculosis

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Introduction

In the past few years, the number of people infected with pulmonary tuberculosis has increased.[1]Today, it kills thousands in Africa alone, millions worldwide and the numbers keep on increasing due to COVID-19 (WHO, 2021). Moreover, resistance to conventional anti tuberculosis recommended drugs is increasing thus rendering more and more difficult to treat this deadly disease.[2] Currently, one third of the world population is infected with various forms of TB,[3] and each year 2-3 million people of the world die from TB infection or its complications.[4] Based on an estimate, nearly one billion people were affected by the disease between 2000 and 2020.[5]

HIV increases the risk of developing active TB, and this makes the treatment of TB difficult for the patients.[6] The treatment is often complicated by the development of resistant strains due to nonadherence to treatment, treatment abandonment because of the multiple side effects, alcoholism, shortage in tuberculostatic drugs etc. Unexpected drug resistance is one of the challenges[7] and this study aimed at improving response to existing regimens through *Artemisia afra* preparation supplementation of the WHO approved treatment regimens. In one of our recent papers, we reported that *Artemisia* plants especially *Artemisia Afra* may promote cure of tuberculosis.[8]

We demonstrated this in DR Congo and Burundi on 50 patients. This study therefore aimed at proving that given as a complementary treatment to conventional regimens (WHO approved national program RHZE: RIFAMPICIN, ISONIAZID, PYRAZINAMIDE, ETHAMBUTOL), *Artemisia Afra* infusions were able to speed up recovery thus shortening treatment duration, minimizing side effects and sickness burdens (lack of energy). Encouraged by what we observed in the previous study on 50 patients, we decided to increase the number to 102 patients and to conduct the new study under a strict national protocol and using a reputed medical institution for purpose of proper participants follow-up, monitoring, and documentation. This study shows that given with the WHO (World Health Organization) approved drugs, *Artemisia Afra* Infusions can shorten the symptoms and treatment duration thus avoiding resistance and speed up recovery both clinically and Para clinically.[9]

Materials and methods

Study design

This study was nested into a one arm of clinical trial (102 adults) of *Artemisia Afra* combined to the National Tuberculosis Plan.

Study site and sample size

The clinical studies took place within Heal Africa Hospital, 111 Lyn Lusi Boulevard, Goma, North Kivu Province, DRC a tertiary hospital (equipped by an international recognized laboratory and 40 medical specialists in internal medicine, laboratory, radiology) in Goma, DRC on 102 adult patients who tested positive on the Ziehl Neelsen sputum test.

Enrolment, follow up and measurements

All the 102 patients with positive smears were enrolled. The *artemisia afra* infusion was prepared everyday by a qualified Pharmacist by infusing 10 gm of the leaves & twigs mixture into 1 liter of water at 97°C and each patient was given to drink 330mls given 3 times daily with every meal rich in protein. Patients were hospitalized for the 5 first days or longer as per the Treating Doctor informed decision. After that they were discharged but had to report to the hospital for additional sputum smear on Day 10,15,20,25 and 30 or until the sputum smear was Ziehl Neelsen negative. While in hospital the patients were on 24-hour monitoring by nurses and were given 3 meals daily. Additional laboratory levels (ESR, CRP) and the Xray: D0 D30 were monitored according to protocol that had been approved by the DRC Research Ethics Committee. It is important to note that the patients continued to receive their drugs (WHO approved pills) and that the *Artemisia Afra* infusion was delivered to them by an agent after its preparation at the Heal Africa Pharmacy daily. In fact, the hospital nurse noted in the appropriate registry that the patient took all his medications and swallowed the Artemisia infusion according to the DOT (Directly Observed Therapy) system for TB.

Results and discussion

We enrolled 102 patients in total and 95(93.2%) of them had complete symptom resolution in that they had their main symptoms: fever, cough, weakness disappear and their sputum test negative within the protocol deadline (30 days from admission and initiation of treatment). It usually takes several weeks to achieve complete symptoms resolution and longer for negative sputum tests. The other 7(6.8%) recovered between Day 31 and Day 52.

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One patient however deserves a particular attention. Patient no 032 IK HA 03.22 was admitted at Heal Africa Hospital on March 13, 2022, with the following symptoms: persistent diurnal and nocturnal cough, asthenia, aphonia, extreme weight loss and lack of appetite (see attached picture). He was put on IVs and oxygen therapy under strict 24 hours monitoring. The next morning, he was given Artemisia infusion and the WHO tablets and after 3 days, there was a significant clinical improvement. He recuperated his appetite and started feeling much better. It is important to note that this patient has been on WHO tablets for 77 days and that his health had significantly deteriorated prior to his enrolment in our study. It is also important to note that his lungs x-ray improved significantly after treatment as we can see on the attached X-ray reports. Finally, as we can notice on the Ziehl Neelsen sputum smear there were several KOCH bacilli on Day 0 and none on Day 30(see picture below).

Other 3 patients (pictures below) also drastically recuperated and had their symptoms resolved quickly and they also added weight in less than 30 days (45 kgs to 55 kgs). Data showing unprecedent health improvement are available for the other 99 patients if requested.

Patient 03 IK HA 03.22

Day 0 Day 30

Day 0 Day 30

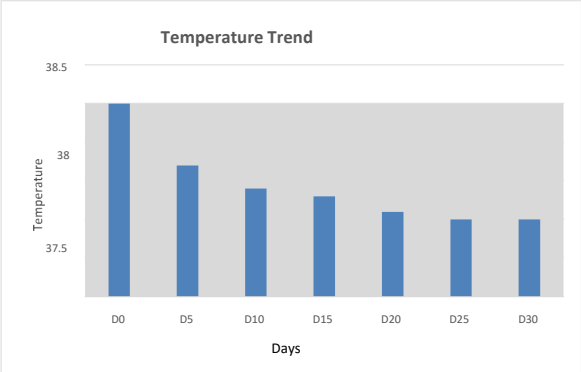
Day 0 Day 30

Day 0 Day 30

The findings are summarized in the tables and figures below

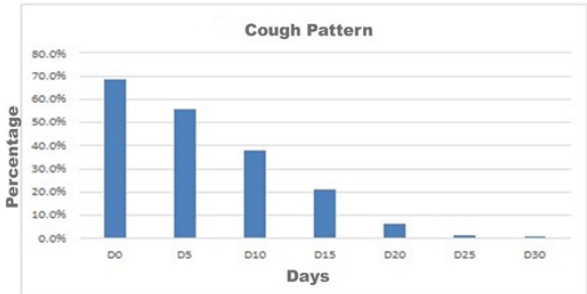
Temperature trend

Days	Results
D0	38
D5	37.2
D10	36.9
D15	36.8
D20	36.6
D25	36.5
D30	36.5



Diurnal cough trend

Days	Values %
D0	68.50%
D5	55.60%
D10	38.00%
D15	21.30%
D20	6.50%
D25	1.20%
D30	0.90%



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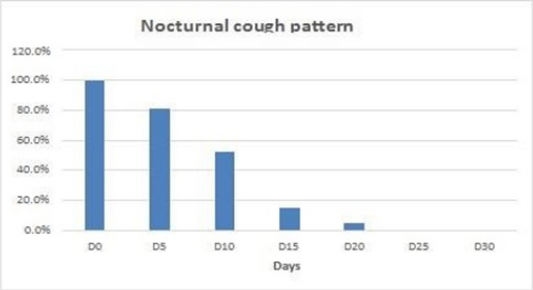
Nocturnal cough trend

Days	Values
D0	100.00%
D5	80.60%
D10	51.90%
D15	14.80%
D20	5.20%
D25	0.30%
D30	0.00%



ESR trend

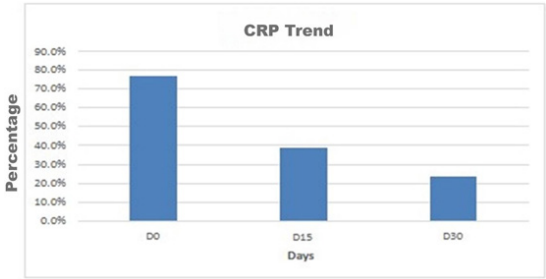
For our study, the following table shows the ESR mean value trend during the treatment

Days	Values
D0	76.60%
D15	38.70%
D30	23.50%

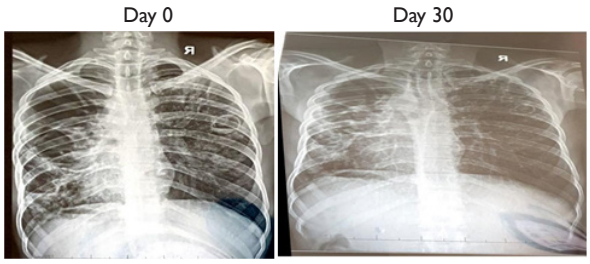
CRP

Although very expensive and mostly unavailable in most poor countries, we run this lab test to ensure that we will be closely following its trend as an additional tool for diagnosis and treatment follow up. Values higher than 10 mg/Liter suggest an inflammation.[10] This table shows the CRP trend for our patients. It is interesting to note that the CRP value is decreasing as the healing progress is taking place. According to the approved protocol it is the Ziehl Neelsen test that was the sole criterium to confirm that the patients were healed.

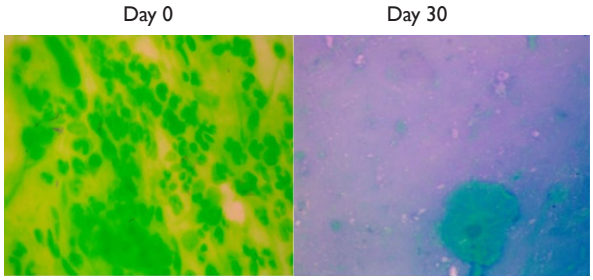
Days	Values
D0	76.60 mg/Liter
D15	38.70 mg/Liter
D30	23.50 mg/Liter



X ray for patient no 032 IK HA 03.22



Slide for patient no. 032 IK HA 03.22 On Day 0 and Day 30



Review: possible explanations for the strong antimycobacterial properties of *Artemisia afra*

The key questions are of course why *Artemisia* plants show this unsuspected efficacy against tuberculosis and why *Artemisia afra* which does not contain artemisinin has a significantly higher efficacy.

Herbal medicine and *Artemisia* against tuberculosis

A study from India finds that among some 10 medicinal herbs there are only two with antimycobacterial effects, *Artemisia nilagirica-vulgaris* and *Murraya koeningii*. Others like *Azadirachta indica*, *Moringa oleifera* have no effect. The ethanolic extract of *A nilagirica* was found to kill intracellular mycobacteria. In the Artemisia family the anti-TB properties of *Artemisia afra* are described in several papers.[14,15] *Artemisia abyssinica* showed *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains.[16-18]

A. absinthium was tested against tuberculosis on laboratory animals with promising results.[19] *Artemisia afra* has been traditionally used for respiratory disorders such as coughs, colds, whooping cough, bronchitis, asthma, and pneumonia.[20] There are so far no papers on similar properties documented for *Artemisia annua*. The antimalarial agent, artemisinin itself is not known to be active against tuberculosis. There may even be negative interactions with other molecules. Antagonism between artemisinin based antimalarial drugs and tuberculosis treatment has been documented.[21]

Rifampicin intake simultaneously with Coartem lowers the AUC of artemether by 83% and lumefantrine by 84%. Similar antagonisms have been noticed for Efavirenz and Nevirapine which are often used indiscriminately against HIV and tuberculosis. The Makerere University found that co-administration of Coartem with Efavirenz resulted in reduction in artemether, DHA, lumefantrine exposure.[22] The mechanism for the action of *Artemisia* against mycobacterium is not fully understood. It is likely that the flavone luteolin plays a key role in the case of *Artemisia afra*.[23-25] The effects of thymol and scopoletin have been studied.[26,27]

A thesis at the University of Fès studied the bactericidal action of essential oils of Thyme, Rosemary and *Artemisia* and found that all three were active against Mycobacteria. The monoterpenes thymol, carvacrol and eugenol were the strongest.[28] *Artemisia* plants are rich in arginine which generates the strong oxidant nitric oxide NO. NO has an important role in the immune defense against tuberculosis. In pulmonary TB patients' low levels of NO are found in the exhaled air and low levels of NO metabolites in urine.[29-34] Stems of medicinal plants contain more arginine, a strong antimalarial, than the leaves which often contain none. For this reason, it is vitally important not to discard stems and twigs from dried *Artemisia* plants.[35] Nitrate content of various parts of a plant differ.[36] Indeed, the vegetable organs can be listed by decreasing nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed.

Artemisia plants are also rich in polyunsaturated fatty acids (PUFA). Arachidonic acid activates PGE2 in macrophages infected by *Mycobacterium tuberculosis.* This promotes plasma cell repair. If the PGE2 concentration is low, this leads to macrophage and granuloma rupture i.e., necrosis and spreading of the encapsulated mycobacteria.[37]

A study in 2006 confirmed that arachidonic acid is highly mycobactericidal in a concentration- and pH-dependent manner *in vitro.* Recent data from animal models have demonstrated that arachidonic acid stimulates phagosome maturation in infected macrophages and promotes apoptosis.[38-40]

A research team in Nigeria[41] examined the use of medicinal plants in the treatment of tuberculosis. Eighty randomly selected questionnaires were administered to traditional healers, herbs traders, farmers and civil servants in the Local Government and were interviewed on the use of forest plants in the treatment of tuberculosis. This study has proven that local people have a wealth of knowledge that needs to be the focal point in pharmaceutical research.

It is very encouraging to find on internet the following project in Ghana, project funded by WHO.[42] For several of the herbs involved in this project the inhibition of mycobacteria is equivalent or better than for Pefloxacin and/or Miconazole. But the most encouraging are the conclusions of this WHO sponsored project: A single herb is never a single compound but a group of compounds which potentiate each other or create synergy. The use of an herb or herbal cocktail would simulate combination therapy, which may prevent, or at least delay the development of microbial resistance.

Malaria, hemozoin, and tuberculosis

Already 30 years ago it was known that human monocytes which have ingested hemozoin are unable to neutralize pathogenic bacteria, fungi, and tumor cells, and that macrophage responses decline during human and animal malaria. Hemozoin (Hz) is a biocrystal synthesized by Plasmodium and other blood-feeding parasites to avoid the toxicity of free heme derived from the digestion of hemoglobin during invasion of the erythrocytes.[43,44]

Hemozoin (malaria pigment) is found in many tissues during malaria infections. In mice that have self-cured from *Plasmodium yoelii* and *Plasmodium chabaudi* infections, liver hemozoin concentration and total content decreased for 6-9 months after parasite clearance. However, both spleen hemozoin concentration and total hemozoin content increased dramatically during this period. Thus, hemozoin or hemozoin-laden macrophages continue to accumulate in murine spleens for at least several months after malaria parasitemia becomes undetectable.[45]

This is well described in a recent paper. Plasmodium infection impairs host immunity to diverse bacteria, including *S. pneumoniae*, through multiple effects on innate immunity, and that a parasite-specific factor (Hz+bound bioactive molecules) directly contributes to Plasmodiuminduced suppression of antibacterial innate immunity biomolecules. Due to the highly amphiphilic nature of hemozoin these biomolecules easily adsorb to hemozoin crystals.

There remain important knowledge gaps that impede treatment of bacteria co-infections during malaria. Few studies have looked directly at the interactions between innate immune cells and Hz in the context of *in vivo* bacterial infection. Malaria and tuberculosis (TB) endemic regions overlap considerably, especially in sub-Saharan Africa. Although it is very likely that coinfections occur in these regions, not much is known about malaria-TB co-infections in humans, and how the interplay between these two infections might affect the prognosis of coinfected individuals.

Hemozoin isolated from Plasmodium infected mice containing naturally associated bioactive molecules, such as host and parasite-derived proteins and lipids significantly impaired the ability of splenic phagocytes to control growth of intracellular bacteria. Following erythrocyte rupture, Hz is released into circulation and engulfed by phagocytic cells resulting in deposition in tissues and organs such as spleen, liver, brain, lungs, and bone marrow.

In their study with *Plasmodium yoelii* infected mice, they found a decreased survival of infected mice following *Salmonella pneumonia* infection. Bacterial burdens trended higher in the lungs and were significantly higher in both the blood and spleen. These data demonstrate that Plasmodium infection also impairs control of bacteremia, and that the Plasmodium- driven susceptibility is prevalent beyond clearance of infected RBCs from peripheral circulation. This impaired antimicrobial functions of innate immune cells from African children with uncomplicated malaria was found to last up to 8 weeks after antimalarial treatment.

Production of reactive oxygen species (ROS) is an important antibacterial effector mechanism used by phagocytic cells that is necessary for immunity to a multitude of bacterial pathogens. The results of this study clearly identify impairment of ROS production in neutrophils as a potential mechanism by which Plasmodium suppresses antibacterial innate immunity during systemic bacterial infections. An association between pulmonary pathology (SARS) and the levels of Hz deposition was also found. The impairment of ROS is not surprising, because during the parasite's erythrocytic life cycle stages, it produces hemozoin to avoid oxidative stress.[46]

A study in Portugal confirmed that repeated malarial episodes will lead to increased Hz deposition in host organs with potential detrimental effects. If Hz impairs cellular functions, such as phagocytosis and oxidative burst, the ability to kill intracellular bacteria might be impaired. It was also observed that hemozoin-containing macrophages participated in granuloma formation in response to tuberculosis infection. This might prove relevant in the context of a tuberculosis infection by the virulent strain M. tuberculosis, in which tighter control by immune cells is necessary to prevent tuberculosis dissemination.

They found that during incubation of synthetic hemozoin (sHz) with whole blood monocytes and granulocytes readily absorb hemozoin. PMBC cells loaded with hemozoin have a lower phagocytic capacity. The Portuguese study also confirms the reduction of ROS production. The mechanism by which hemozoin ingestion leads to impairment of phagocytosis is not known. However, inhibition of ROS production

by hemozoin ingestion seems to be associated to production of lipid peroxidation derivatives, which promote oxidation and damage of essential components of the oxidative response.

They were also impressed by the long residence time of hemozoin in the body. Considering that the average life span of a mouse is around 850 days, it is impressive that hemozoin could still be detected in mouse organs 280 and 140 days. This represents approximately a quarter of the life span of a mouse. If the same happens in humans and considering that in malaria endemic regions, such as sub-Saharan Africa, the same individual is likely to get infected several times throughout life, then it is likely that the amount of hemozoin accumulated in organs is enormous. If intracellular hemozoin in these organs consistently contributes to impairment of functions of these cells, then affected individuals will potentially have a proportion of immune cells that respond to other pathogens at suboptimal levels.[47]

The causal relationship between hemozoin and lung inflammation was investigated[48] and confirmed by injecting *P. falciparum*-derived hemozoin intravenously into malaria-free mice. The extensive work done by the University of Al-Quds in Palestine on beta-hematin inhibition by Artemisia and other plants may open a large array of possibilities to reduce the hemozoin load in the human body.[49]

Diabetes and tuberculosis

The international community has also been alerted on the Diabetes and tuberculosis coepidemic.[50] Diabetes triples the risk of developing tuberculosis (TB). Consequently, rates of TB are higher in people with diabetes than in the general population, and diabetes is a common comorbidity in people with TB. Diabetes can worsen the clinical course of TB, and TB can worsen glycemic control in people with diabetes. The excess vulnerability to TB disease in people with diabetes is mainly related to altered immune response to TB infection because of high blood sugar due to undiagnosed or poorly controlled diabetes. A Lancet study in 2009 had confirmed the convergence of the two epidemics.[51]

In 1934 already, this link had been discovered in Boston MA.[52] If diabetes may lead to tuberculosis, tuberculosis may also lead to diabetes.[53-55] Two retrospective cohort studies of patients with pulmonary tuberculosis in Maryland, USA, have shown a 6.5 times increased risk of death in diabetes patients.[56] Traditional medicine may provide some hope and the results obtained against diabetes in Maniema, Congo with *Artemisia* plant infusions are encouraging.[57]

Iron and tuberculosis

Three diseases at least are caused by mycobacteria: leprosy, tuberculosis and Buruli ulcer. Iron is a prerequisite for the growth of mycobacteria. Clinical studies in Africa have established a correlation between dietary iron overload and enhanced risk of death from tuberculosis.[58-62] The incidence of tuberculosis has increased markedly over the last decade. Dietary iron overload affects up to 10% of adults in rural populations and is characterized by heavy iron deposition both in parenchymal cells and in macrophages.

The incidence of infections was studied in 137 iron-deficient Somali nomads, 67 of whom were treated with placebo and 71 with iron. Seven episodes of infection occurred in the placebo group and 36 in the group treated with iron; these 36 episodes included activation of preexisting malaria, brucellosis, and tuberculosis. This difference suggested that host defense against these infections was better during iron deficiency than during iron repletion. Iron deficiency among Somali nomads may be part of an ecological compromise, permitting optimum co-survival of host and infecting agent.

It is deplorable that already in 1926 Strachan AS (MD thesis, Glasgow) had established that the odds of death from tuberculosis in South Africa were 16.9 times higher in people with a splenic iron overload.[63] In a tragic attempt to rectify what was perceived as a debilitating iron deficiency in patients in Somalia, iron supplementation was actually found to promote the development of active tuberculosis.[64]

Chelation of iron may reduce *M. tuberculosis* replication, restore host defense mechanisms and it could constitute an application in the prevention and treatment strategies where both iron overload and tuberculosis are prevalent. Proanthocyanins are strong iron chelators and *Artemisia* plants are rich in condensed tannins. Chelation of zinc with quercetin and luteolin has been studied recently in Greece. *Artemisia afra* is much richer in luteolin than *Artemisia annua.*[65,66]

Lactoferrin is a strong chelator of iron. This explains why it may provide protection and decrease immunopathology of mycobacteria. It can be supplied by milk products (whey for example), but it is also produced by mucosal epithelial cells in various mammalian species including humans, particularly in nasal and bronchial secretions.[67]

A study from Texas[68] by lactoferrin treatment, intravenously injected or orally administered, showed statistically significant fewer and smaller granulomas than control. Of importance, the protective proinflammatory mediators were not statistically diminished by lactoferrin treatment.

Hepcidin is an antimicrobial peptide produced by the liver in response to inflammatory stimuli and iron overload. Hepcidin regulates iron homeostasis by mediating the degradation of the iron export protein ferroprotein, thereby inhibiting iron absorption from the small intestine and release of iron from macrophages. As hepcidin has been shown to possess direct antimicrobial activity, a study investigated its activity against *M. tuberculosis.* It was found that hepcidin inhibited *M. tuberculosis* growth *in vitro* and caused structural damage to the mycobacteria.[69]

Gallium and tuberculosis

Gallium is a semi metallic element. Its biological actions stem from its ionic radius which is almost the same as that of the ferric iron, whereby it can replace iron in Fe (III)-dependant biological systems and enzymes. Unlike Fe (III), ionic Ga (III) cannot be reduced and when incorporated, it inactivates Fe (III) dependant oxidation and reduction processes, that are necessary for bacterial and mammalian cell proliferation. Incorporated in iron-specific enzymes and proteins it acts like a Trojan horse. Fe metabolism is of high vulnerability for infecting bacteria because they require Fe for growth. Gallium is known to disrupt iron metabolism in *Mycobacteria tuberculosis* residing within human macrophages.

Gallium's anti-infective activity against bacteria and fungi results from disruption of microbial iron utilization through mechanisms which include gallium binding to siderophores and downregulation of bacterial iron uptake.[70] Gallium markedly inhibits this Fe acquisition by *Mycobacterium tuberculosis* into the macrophages. Biological systems are unable to discriminate between Ga and Fe. But Ga fails to undergo the redox cycling of Fe and the Fedependent metabolic pathways of mycobacteria. This leads to growth inhibition.[71] Gallium is bactericidal for mycobacteria growing within macrophages. It was found that oral administration of gallium salts might be useful as a prophylactic agent against *Mycobacterium avium subsp paratuberculosis* infection in neonatal calves.[72]

Nickel and tuberculosis

There is evidence that nickel is an essential trace element in several animal species, plants and prokaryotic organisms. Nickel appears to be essential for humans, although no data are available concerning nickel deficiency. In most food products, the nickel content is less than 0.5 mg/kg fresh weight or 5 mg/kg dry weight. *Artemisia* plants are metal accumulators. This is well documented in one of our studies in Senegal.[73] An analysis of 7 medicinal plants in Pakistan[74] showed that *Artemisia vulgaris* had the highest concentration of nickel. This is confirmed by a study from Malaysia.[75] *Artemisia vulgaris* contained 23.4 mg/kg and the other 15 plants only an average of 4 mg/kg. This is much higher than the content in green vegetables =.11 mg/kg), potatoes (0.10), fresh fruits (0.03).

High concentrations of nickel are toxic for humans, but low concentrations might also have a beneficial effect. Fumes generated in metal plating operations and containing nickel salts induce IgE which are immunogenic. Gold vapors do not generate this effect.[76,77] Nickel is used in vaccines to increase IgG.[78,79] The genome of the human pathogen *Mycobacterium tuberculosis* encodes an impressive diversity of known and putative metal ion transporters This diversity might enable this obligate intracellular pathogen to efficiently respond to a range of host killing mechanisms, collectively known as nutritional immunity. Although the importance of Ni homeostasis in *Mycobacterium tuberculosis* pathogenesis remains poorly understood, it has been reported that total nickel levels as measured by x-ray fluorescence microscopy fall precipitously inside infected macrophages.[80,81] A study suggested that the exposure to nickel dust has unspecifically activated the macrophages perhaps by increased production of phospholipids.[82]

Silica and tuberculosis

Silica has been documented to cause apoptosis in cells like alveolar macrophages. Apoptosis of alveolar macrophages and granuloma leaves no chance for the mycobacteria hiding inside the now death cells to escape and spread throughout the body. Cell death pathway seems to influence the outcome of infection. In attenuated tuberculosis apoptosis is predominant and in virulent tuberculosis necrosis (Figure 1).

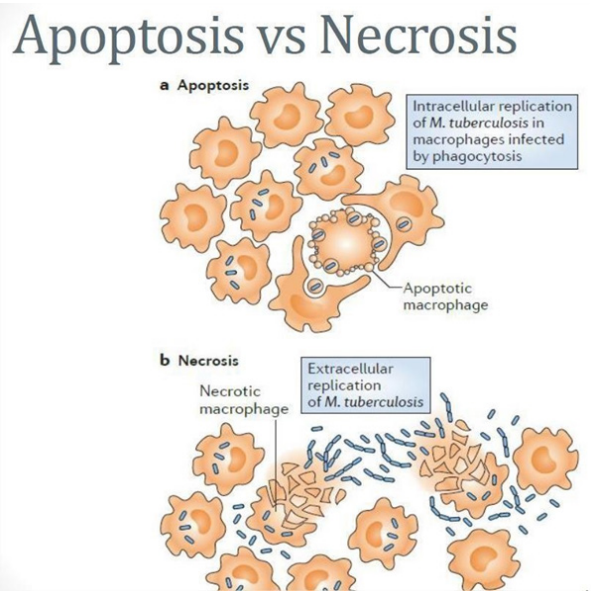


Figure 1 Necrosis versus apoptosis.[83]

In an assay it was found that intratracheal instillation of silica could induce apoptosis in both alveolar and granulomatous cells, and the apoptotic change and subsequent engulfment by macrophage. Death granuloma cannot spread the infection as it happens in necrosis and rupture of the granuloma. Fifty-six days after instillation, morphologically apoptotic cells could be identified in granulomatous cells of lung tissue from silica-instilled rats. In the same trial apoptosis of leukocytes was noticed. Leukocytosis is a well-known marker of symptomatic tuberculosis.[84-86]

Silicosis is a lung disease induced by the inhalation of crystalline silica. Exposure of cultured macrophages to crystalline silica leads to cell death. Time-lapse imaging shows that mouse macrophages avidly bind particles that settle onto the cell surface and that cells also extend protrusions to capture distant particles. Macrophages internalize silica. Eighty percent of macrophages die within 12 hours of silica exposure. The toxicity of silica particles which leads to silicosis, at the same time may be beneficial in a disease like tuberculosis where the silica particles kill the macrophages and granuloma infected by mycobacteria.[87]

Silica released from necrotic macrophages is as cytotoxic as the original preparation. It is suggested that repeated cycles of macrophage killing *in vivo* lead to prophylactic and therapeutic effects of long duration.[88] Cells stimulated by silica nanoparticles release large amounts of reactive oxygen species, tumor necrosis factor-α and nitric oxide. Silica particles also increase production of interleukin-6.[89] In many plants, silica is present in the form of phytoliths in cell walls for their strengthening. Families with high phytolith production are Acanthaceae, Aceraceae, Annonaceae, Asteraceae. *Artemisia tridentata* and *Artemisia spicata* for example contain phytolith.[90]

Phytoliths are present in the glandular trichomes of the plants. Trichomes are fine outgrowths or appendages on plants. Trichomes serve as the plant's phalanx of little shields responsible for the developing protection against fungus and insects.[91] The presence of trichomes is ubiquitous in the genus *Artemisia.* 15 taxa were examined to this effect to be used as taxonomic markers.[92]

Zinc and tuberculosis

Zinc has a pivotal role in the entire immune system fostering resistance to infections by virus, fungi and bacteria. Zinc functions as an antioxidant. It protects cells of damaging effects of oxygen radicals generated during immune activation. But the human body has no zinc storage system, hence, a daily nutritional zinc uptake is necessary. The immune system is strongly impaired by zinc deficiency, predominantly the cell-mediated response by T-lymphocytes. *Artemisia* plants are rich in zinc.[93] A significant reduction in serum zinc levels was noticed in all types of leprosy *(Mycobacterium leprosis).* Conversely, the copper levels were significantly increased.[94]

The Institute Pasteur has shown that the immune cells are capable of mobilizing reserves of heavy metals, especially zinc, to poison microbes. In macrophages that have ingested *M. tuberculosis or E. coli*, the researchers observed a rapid and persistent accumulation of zinc. Zinc, although toxic when ingested in too high quantities, is therefore beneficial for the immune system, particularly because it is used by macrophages to poison microbes. Oral zinc was tried in 15 cases of multibacillary leprosy[95] as an immunostimulant in addition to conventional antileprosy drugs. Results were compared with those in ten similar cases treated with dapsone alone. Cases treated with zinc showed faster clinical improvement. Upgrading occurred in 6 out of 15 patients taking zinc, but in only 1 out of 10 patients in the control group. For pulmonary tuberculosis, a study from Mexico

showed that earlier elimination of bacilli from sputum was associated with improved zinc status and Th1 immune response. The therapeutic effect of vitamin A was less evident. If confirmed in clinical trials, this adjunctive therapy could be used to shorten the amount of time that TB patients are contagious.[96]

The effectiveness and success of antituberculosis therapy is mainly measured by its ability to identify the organism in the sputum. Another research team found that during treatment there was a significant increase in the levels of Zn and a decrease in the Cu/Zn ratio. Serum Zn levels and the Cu/Zn ratio could be used as a valuable laboratory tool for the clinicians to assess response to therapy or effectiveness of the ongoing antituberculosis therapy.[97-99]

Selenium and tuberculosis

Selenium levels are very low in people infected by *Mycobacterium ulcerans* and *M tuberculosis.* In Malawi lower plasma levels of selenium were found in smear-positive tuberculosis patients. This was confirmed by another study: low selenium concentrations, high HIV load and high IL-6 concentrations are associated with anemia in adults with pulmonary tuberculosis.[100-102] Plants from the *Artemisia* family also accumulate many minerals, including selenium, 10 times more than fruits and vegetables.[103] The same accumulator effect has been shown in China for *Artemisia argyi*[102] and the US for *Artemisia ludoviciana.*[104]

Polytherapy with *Artemisia* plants or monotherapy?

Monotherapy with pharmaceutical drugs may be effective for some time, but in most cases, resistance develops rapidly. The best evidence that interactions play a key role and that pure monosubstances are not necessarily the ultimate in therapy were obtained in a Swiss study. This piece of work shows that the isolation of individual ingredients from a plant for the purposes of being able to control them better or for commercial benefit does not always lead to the desired therapeutic objective. The study deals with ursolic acid which is present in *Artemisia* plants.[105]

The Swiss study explored the variability of biological responses from the perspective of sample purity and introduces the concept of purity–activity relationships (PARs) in natural product research. The abundant plant triterpene ursolic acid was selected as an exemplary natural product due to the overwhelming number yet inconsistent nature of its approximate 120 reported biological activities, which include anti-TB potential. In the Swiss study nine different samples of ursolic acid with purity certifications were obtained, and their purity was independently assessed by means of quantitative NMR. Biological evaluation consisted of determining MICs against two strains of virulent *Mycobacterium tuberculosis* and IC50 values in Vero cells.

An inverse correlation of purity and anti-TB bioactivity was demonstrated. The results imply that synergistic effects of ursolic acid and its varying impurities are the likely cause of previously reported antimycobacterial potential. Minute traces of impurities therefore obviously produced a better effect against tuberculosis bacteria. Or, to put it in a nutshell: without impurities, the preparation had no effect (Figure 2)!

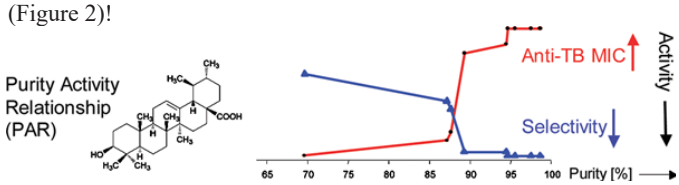


Figure 2 Purity–activity relationships.

Similar results on the purity activity relationship were obtained in another study for triterpenes. Another good example of synergy is the interaction between zinc and arginine. Both components play a role in tuberculosis. Our partners at the Al Quds University in Palestine have found that a zinc-arginine complex strongly inhibits beta-hematin crystallization, like quinine does, but that zinc or arginines alone are not effective (Figure 3).

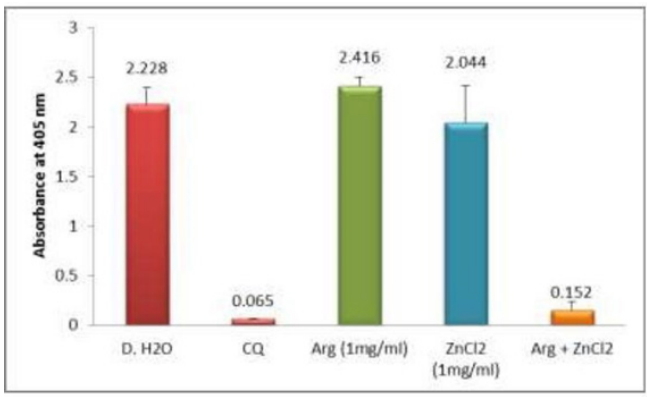


Figure 3 Antimalarial property measured as per beta-hematin inhibition. Only the complex has antimalarial properties, the individual compounds not (Mutaz Akkawi, Al-Quds University, personal communication). CQ (chloroquine) known for its strong beta-hematin inhibition properties is the control.

Conclusion

a. It has been very interesting to notice the rapid improvement of the patients both clinically and Para clinically with the improved laboratory parameters (ESR, CRP, Sputum smear).

b. The results are very encouraging and could be a basis to conduct Randomized Clinical Trial and develop a cheap herbal treatment supplement and with less side effects or toxicities.

c. The authors suggest that the next phase of the study will be a comparison of 50 patients on the WHO approved treatment ALONE versus 50 patients on *Artemisia Afra* and WHO drugs.

d. These novel antimycobacterial results and the advanced synergistic activity observed for the *Artemisia afra* infusion present an opportunity to establish a new and cheap way to address resistant tuberculosis and to speed up recovery for regular tuberculosis with no side effects.

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Conflicts of interest

Authors declare that there is no conflict of interest.

References

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

2. Peter Oh, Pascopella L, Barry PM, et al. A systematic synthesis of direct costs to treat and manage tuberculosis disease applied to California. *BMC Res Notes*. 2017;10(1):434.

3. Diel R, Vandeputte J, de Vries G, et al. Costs of tuberculosis disease in the European Union: a systematic analysis and cost calculation. *European Respiratory Journal*. 2014;43(2):554–565.

4. Jérôme Munyangi, Pierre Lutgen. Artemisia afra cures Buruli ulcer with high efficacy. *Malaria world*. 2017;11:24.

5. Singh KJ, Ahluwalia G, Sharma SK, et al. Significance of haematological manifestations in patients with tuberculosis. *J Assoc Physicians India*. 2001;49:788, 790–794.

6. Olaniyi JA, Aken'Ova YA. Haematological profile of patients with pulmonary tuberculosis in Ibadan, Nigeria. *Afr J Med Kenova Sci*. 2003;32(3):239–242.

7. Martins C, de Castro Gama AC, Valcarenghi D, et al. Markers of acute--phase response in the treatment of pulmonary tuberculosis. *J Bras Patol Med Lab*. 2014;50(6):428–433.

8. Ding RD, Zhang HJ. Effect of linezolid on serum PCT, ESR, and CRP in patients with pulmonary tuberculosis and pneumonia. *Medicine (Baltimore)*. 2018;97(37):e12177.

9. Kumar Abhishek. Survey of Changes in the Erythrocyte Sedimentation Rate at the Different Stages of Therapy by Westergren Method in the Patients of Pulmonary Tuberculosis in the Malwa Region of Madhya Pradesh. *International Journal of Physiology*. 2019;7(4):1–5.

10. Rohini K, Surekha Bhat M, et al. Assessment of Hematological Parameters in Pulmonary Tuberculosis Patients. *Indian J Clin Biochem*. 2016;31(3):332–335.

11. Naik SK, Mohanty S, Padhi A, et al. Evaluation of antibacterial and cytotoxic activity of Artemisia nilagirica and Murraya koenigii leaf extracts against mycobacteria and macrophages. *BMC Complement Altern Med*. 2014;14:87.

12. M van de Venter, M Pruissen et al. In vitro anti-HIV and -TB activities of Annona muricata and Artemisia afra extracts. *Planta Med*. 2014;80:P1L29.

13. Gemechu A, Giday M, Worku A, et al. In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis strains. *BMC Complement Altern Med*. 2013;13:291.

14. Uba A, Ibrahim K, Makinde AA. In vitro inhibition of Mycobacterium smegmatis and Mycobacterium tuberculosis by some Nigerian medicinal plants. *East and Central African Journal of Pharmaceutical Sciences*. 2003;6(1):15–19.

15. Masoko P, Nxumalo KM. Validation of antimycobacterial plants used by traditional healers in three districts of the Limpopo province (South Africa). *Evid Based Complement Alternat Med*. 2013;2013:586247.

16. Hojageldiyev T, Bolmammedov Y, Gurbanaliyev S. Antimycobacterial activity of ethanolic extract of Artemisia absinthium L. *World Scientific News*. 2019;119:224–230.

17. Buwa LV, Afolayan AJ. Antimicrobial activity of some medicinal plants used for the treatment of tuberculosis in the Eastern Cape Province, South Africa. *African Journal of Biotechnology*. 2009;8(23):6683–6687.

18. Lamorde M, Byakika-Kibwika P, Mayito J, et al. Lower artemether, dihydroartemisinin and lumefantrine concentrations during rifampicin--based tuberculosis treatment. *AIDS*. 2013;27(6):961–965.

19. Byakika-Kibwika P, Lamorde M, Mayito J, et al. Significant pharmacokinetic interactions between artemether/lumefantrine and efavirenz or nevirapine in HIV-infected Ugandan adults. *J Antimicrob Chemother*. 2012;67(9):2213–2221.

20. Singh DK, Fatima S. *Luteolin enhances antibiotic treatment of Mycobacterium tuberculosis infection by promoting central memory T lymphocyte responses*. Conference paper: Keystone Symposia Global Health Series: Tuberculosis Co- Morbidities and Immunopathogenesis (B6-2016). Keystone Resort, Keystone, Colorado, USA; 2016.

21. Araujo RC, Neves FA, Formagio AS, et al. Evaluation of the anti-mycobacterium tuberculosis activity and in vivo acute toxicity of Annona sylvatic. *BMC Complement Altern Med*. 2014;14:209.

22. Muganga R. *Luteolin levels in selected folkloric preparations and the bioavailability of luteolin from Artemisia afra aqueous extract in the vervet monkey*. Thesis: University of the Western Cape; 2004. 14 p.

23. Brooks C. Chemotherapy of tuberculosis; thymol in experimental tuberculosis in the guinea pig. *Fed Proc*. 1946;5(1 Pt 2):168.

24. Okhale SE, Oladosu P, Orishadipe AT, et al. Identification of Thymol as an Antitubercular Agent from Ocimum gratissimum Leaf Essential Oil. *American Chemical Science Journal*. 2015;9(2):1–6.

25. Rhayour K. *Study of the mechanism of the bactericidal action of essential oils on Esherichia coli, Bacillus subtilis and on Mycobacterium phlei and Mycobacterium fortuitum*. Thesis: Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar Mahraz, Fez; 2002.

26. Jamaati H, Mortaz E, Pajouhi Z, et al. Nitric Oxide in the Pathogenesis and Treatment of Tuberculosis. *Front Microbiol*. 2017;8:2008.

27. Idh J. *The role of NO in host defence against Mycobacterium tuberculosis*. Thesis: Linköping University Medical Dissertations; 2012. 92 p.

28. Schön T. *Nitric Oxide in Tuberculosis and Leprosy*. Linköping University Medical Dissertations, No.749; 2002.

29. Yang CS, Yuk JM, Jo EK. The role of nitric oxide in mycobacterial infections. *Immune New.* 2009;9(2):46–52.

30. Nozaki Y, Hasegawa Y, Ichiyama S, et al. Mechanism of nitric oxide-dependent killing of Mycobacterium bovis BCG in human alveolar macrophages. *Infect Immun*. 1997;65(9):3644–3647.

31. Schön T, Elias D, Moges F, et al. Arginine as an adjuvant to chemotherapy improves clinical outcome in active tuberculosis. *European Respiratory Journal*. 2003;21(3):483–488.

32. Hasan HM, Farage M, Saad EK. Amino Acid content of leaves and stems for three types of herbal plants. *World J Chemistry*. 2014;9(1):15–19.

33. Qiu W, Wang Z. Nitrate accumulation in leafy vegetables and its relationship with water. *J Soil Sci Plant Nutr*. 2014;14(4):761–768.

34. Munyangi J, Lutgen P. Artemisia plants, arachidonic and other polyunsaturated fatty acid. *Malariaworld J*. 2020;11:3.

35. Vandal OH, Gelb MH, Ehrt S, et al. Cytosolic phospholipase A2 enzymes are not required by mouse bone marrow-derived macrophages for the control of Mycobacterium tuberculosis in vitro. *Infect Immun*. 2006;74(3):1751–1756.

36. Jordao L, Lengeling A, Bordat Y, et al. Effects of omega-3 and -6 fatty acids on Mycobacterium tuberculosis in macrophages and in mice. *Microbes Infect*. 2008;10(12-13):1379–1386.

37. Yuhas Y, Azoulay-Alfaguter I, Berent E, et al. Rifampin inhibits prostaglandin E2 production and arachidonic acid release in human alveolar epithelial cells. *Antimicrob Agents Chemother*. 2007;51(12):4225–4230.

38. Faleyimu OI, Akinyemi O, Adejoba OR. Herbal solution to the treatment of tuberculosis infection in Kaduna south local government, Kaduna, Nigeria. *Journal of Environmental Extension*. 2009;8.

39. Addo P, Owusu E, Adu Addai B, et al. In-vitro susceptibility of Mycobacterium ulcerans to herbal preparations. *The Internet Journal of Tropical Medicine*. 2007;4(2).

40. Schwarzer E, Turrini F, Ulliers D, et al. Impairment of macrophage functions after ingestion of Plasmodium falciparum-infected erythrocytes or isolated malarial pigment. *J Exp Med*. 1992;176(4):1033–1041.

41. Turrini F, Schwarzer E, Arese P. The involvement of hemozoin toxicity in depression of cellular immunity. *Parasitol Today*. 1993;9(8):297–300.

42. Levesque MA, Sullivan AD, Meshnick SR. Splenic and hepatic hemozoin in mice after malaria parasite clearance. *J Parasitol*. 1999;85(3):570–573.

43. Harding CL, Villarino NF, Valente E, et al. Plasmodium Impairs Antibacterial Innate Immunity to Systemic Infections in Part Through Hemozoin-Bound Bioactive Molecules. *Front Cell Infect Microbiol*. 2020;10:328.

44. Deroost K, Tyberghein A, Lays N, et al. Hemozoin induces lung inflammation and correlates with malaria-associated acute respiratory distress syndrome. *Am J Respir Cell Mol Biol*. 2013;48(5):589–600.

45. Frita, Rosangela MRC. *Malaria and tuberculosis co-infection: role for hemozoin immunosuppression*. Teses de Doutoramento. Universidade de Lisboa, Faculdade de Medicina; 2014.

46. Deroost K, Tyberghein A, Lays N, et al. Hemozoin induces lung inflammation and correlates with malaria-associated acute respiratory distress syndrome. *Am J Respir Cell Mol Biol*. 2013;48(5):589–600.

47. Akkawi M, Jaber S, Abu-Remeleh Q, et al. Investigations of Artemisia Annua and Artemisia Sieberi Water Extracts Inhibitory Effects on B-Hematin Formation. *Med Aromat Plants*. 2014;3:150.

48. Declaration at a Summit in Bali, organised by the Indonesian Ministry of Health. The Union and World Diabetes Foundation; 2015.

49. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis*. 2009;9(12):737–746.

50. Root H. The Association of Diabetes and Tuberculosis. *N Eng J Med*. 1934;210:78–92.

51. Yorke E, Atiase Y, Akpalu J, et al. The Bidirectional Relationship between Tuberculosis and Diabetes. *Tuberc Res Treat*. 2017;2017:1702578.

52. Nichols GP. Diabetes among young tuberculous patients; a review of the association of the two diseases. *Am Rev Tuberc*. 1957;76(6):1016–1030.

53. Morsy AM, Zaher HH, Hassan MH, et al. Predictors of treatment failure among tuberculosis patients under DOTS strategy in Egypt. *EMHJ-Eastern Mediterranean Health Journal*. 2003;9(4):689–701.

54. Oursler KK, Moore RD, Bishai WR, et al. Survival of Patients with Pulmonary Tuberculosis: Clinical and Molecular Epidemiologic Factors. *Clin Infect Dis*. 2002;34(6):752–759.

55. Munyangi J, Idumbo M, Mupenda B, et al. Five case reports on treatment of diabetes by Artemisia annua and Artemisia afra herbal tea. *Pharm Pharmacol Int J*. 2020;8(2):79–85.

56. Gordeuk VR, McLaren CE, MacPhail AP, et al. Associations of iron overload in Africa with hepatocellular carcinoma and tuberculosis: Strachan's 1929 thesis revisited. *Blood*. 1996;87(8):3470–3476.

57. Moyo VM, Gangaidzo IT, Gordeuk VR, et al. Tuberculosis and iron overload in Africa: a review. *Cent Afr J Med*. 1997;43(11):334–339.

58. De Voss JJ, Rutter K, Schroeder BG, et al. Iron acquisition and metabolism by mycobacteria. *J Bacteriol*.1999;181(15):4443–4451.

59. Kochan I. The role of iron in bacterial infections, with special consideration of hosttubercle bacillus interaction. *Curr Top Microbiol Immunol*. 1973;60:1–30.

60. Boelaert JR, Vandecasteele SJ, Appelberg R, et al. The Effect of the Host's Iron Status on Tuberculosis. *J Infect Dis*. 2007;195(12):1745–1753.

61. Strachan AS. *Hemosiderosis and Haemochromatosis in South African Natives with a Comment on the Etiology of Haemochromatosis*. MD Thesis: Glasgow, UK, University of Glasgow; 1929.

62. Murray MJ, Murray AB, Murray MB, et al. The adverse effect of iron repletion on the course of certain infections. *Br Med J*. 1978;2(6145):1113–1115.

63. Cronjé L, Edmondson N, Eisenach KD, et al. Iron and iron chelating agents modulate Mycobacterium tuberculosis growth and monocyte-macrophage viability and effector functions. *FEMS Immunol Med Microbiol*. 2005;45(2):103–112.

64. Primikyri A, Mazzone G, Lekka C, et al. Understanding zinc (II) chelation with quercetin and luteolin: a combined NMR and theoretical study. *J Phys Chem B*. 2015;119(1):83–95.

65. Welsh KJ, Hwang SA, Boyd S, et al. Influence of oral lactoferrin on Mycobacterium tuberculosis induced immunopathology. *Tuberculosis (Edinb)*. 2011;91 Suppl 1:S105–S113.

66. Actor JK. Lactoferrin: A Modulator for Immunity against Tuberculosis Related Granulomatous Pathology. *Mediators Inflamm*. 2015;2015:409596.

67. Sow FB, Florence WC, Satoskar AR, et al. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. *J Leukoc Biol*. 2007;82(4):934–945.

68. Chitambar CR. Gallium and its competing roles with iron in biological systems. *Biochim Biophys Acta*. 2016;1863(8):2044-2053.

69. Olakanmi O, Britigan BE, Schlesinger LS. Gallium disrupts iron metabolism of mycobacteria residing within human macrophages. *Infect Immun*. 2000;68(10):5619–5627.

70. Monk CS, Sweeney RW, Bernstein LR, et al. Serum and tissue concentrations of gallium after oral administration of gallium nitrate and gallium maltolate to neonatal calves. *Am J Vet Res*. 2016;77(2):151–155.

71. Alassane T, Mouhamadou D, El Hadji Omar GP, et al. Characterization of element and mineral content in Artemisia annua and Camellia sinensis leaves by handheld X-ray fluorescence. *African Journal of Biotechnology*. 2013;12(26):4179–4186.

72. Ashraf M, Mumtaz S. A study on elemental contents of medicinally important species of Artemisia L. (Asteraceae) found in Pakistan. *Journal of Medicinal Plants Research*. 2010;4(21):2256–2263.

73. Bath R, Kiran K, Arun AB, et al. Determination of Mineral Composition and Heavy Metal Content of Some Nutraceutically Valued Plant Products. *Food Anal Methods*. 2010;3:181–187.

74. Novey HS, Habib M, Wells ID. Asthma and IgE antibodies induced by chromium and nickel salts. *J Allergy Clin Immunol*. 1983;72(4):407–412.

75. Shirakawa T, Kusaka Y, Morimoto K. Specific IgE antibodies to nickel in workers with known reactivity to cobalt. *Clin Exp Allergy*. 1992;22(2):213–218.

76. Fischer NO, Blanchette CD, Chromy BA, et al. Immobilization of His-tagged proteins on nickel-chelating nanolipoprotein particles. *Bioconjug Chem*. 2009;20(3):460–465.

77. Marques Neto LM, Kipnis A, Junqueira-Kipnis AP. Role of Metallic Nanoparticles in Vaccinology: Implications for Infectious Disease Vaccine Development. *Front Immunol*. 2017;8:239.

78. Reyes-Caballero H, Lee CW, Giedroc DP. Mycobacterium tuberculosis NmtR harbors a nickel sensing site with parallels to Escherichia coli RcnR. *Biochemistry*. 2011;50(37):7941–7952.

79. Campbell DR, Chapman KE, Waldron KJ, et al. Mycobacterial cells have dual nickel-cobalt sensors: sequence relationships and metal sites of metal-responsive repressors are not congruent. *J Biol Chem*. 2007;282(44):32298–32310.

80. Jarstrand C, Lundborg M, Wiernik A, et al. Alveolar macrophage function in nickel dust exposed rabbits. *Toxicology*. 1978;11(4):353–359.

81. Silva Miranda M, Breiman A, Allain S, et al. The tuberculous granuloma: an unsuccessful host defence mechanism providing a safety shelter for the bacteria? *Clin Dev Immunol*. 2012;2012:139127.

82. Shen HM, Zhang Z, Zhang QF, et al. Reactive oxygen species and caspase activation mediate silica-induced apoptosis in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol*. 2001;280(1):L10–L17.

83. Leigh J, Wang H, Bonin A, et al. Silica-induced apoptosis in alveolar and granulomatous cells in vivo. *Environ Health Perspect*. 1997;105(Suppl 5):1241–1245.

84. McCabe MJ Jr. Mechanisms and consequences of silica-induced apoptosis. *Toxicol Sci*. 2003;76(1):1–2.

85. Gilberti RM, Joshi GN, Knecht DA. The phagocytosis of crystalline silica particles by macrophages. *Am J Respir Cell Mol Biol*. 2008;39(5):619–627.

86. Allison AC, Harington JS, Birbeck M. An examination of the cytotoxic effects of silica on macrophages. *J Exp Med*. 1966;124(2):141–154.

87. Chen Q, Xue Y, Sun J. Kupffer cell-mediated hepatic injury induced by silica nanoparticles in vitro and in vivo. *Int J Nanomedicine*. 2013;8:1129–1140.

88. Blinnikov MS. Phytoliths in plants and soils of the interior Pacific Northwest. *Review of Paleobotany and Palynology*. 2005;135(1-2):71–98.

89. Rostkowska C, Mota CM, Oliveira TC, et al. Si-Accumulation In Artemisia annua Glandular Trichomes Increases Artemisinin Concentration, but Does Not Interfere In the Impairment of Toxoplasma gondii Growth. *Front Plant Sci*. 2016;7:1430.

90. Hayat MQ, Ashraf M, Ajab Khan M, et al. Diversity of foliar trichomes and their systematic implications in the genus Artemisia. *Int J Agric Biol*. 2009;11:542–546.

91. Brisibe EA, Umoren UE, Brisibe F, et al. Nutritional characterisation and antioxidant capacity of different tissues of Artemisia annua L. *Food Chemistry*. 2009;115(4):1240–1246.

92. George J, Bhatia VN, Balakrishnan S, et al. Serum zinc/copper ratio in subtypes of leprosy and effect of oral zinc therapy on reactional states. *Int J Lepr Other Mycobact Dis*. 1991;59(1):20–24.

93. Mathur NK, Bumb RA, Mangal HN, et al. Oral zinc as an adjunct to dapsone in lepromatous leprosy. *Int J Lepr Other Mycobact Dis.* 1984;52(3):331–338.

94. Armijos RX, Weigel MM, Chacon R, et al. Adjunctive micronutrient supplementation for pulmonary tuberculosis. *Salud Publica Mex*. 2010;52(3):185–189.

95. Ciftci TU, Ciftci B, Yis O, et al. Changes in serum selenium, copper, zinc levels and cu/zn ratio in patients with pulmonary tuberculosis during therapy. *Biol Trace Elem Res*. 2003;95(1):65–71.

96. Neyrolles O, Mintz E, Catty P. Zinc and copper toxicity in host defense against pathogens: Mycobacterium tuberculosis as a model example of an emerging paradigm. *Front Cell Infect Microbiol*. 2013;3:89.

97. Shor-Posner G, Miguez MJ, Pineda LM, et al. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2002;29(2):169–173.

98. Arntsen A, Sakhi AK, Kalfoss T, et al. Lower plasma levels of selenium and glutathione in smear-positive tuberculosis patients in Malawi. *Ethiopian J Health Dev*. 2011;25(3):230–232.

99. van Lettow M, West CE, van der Meer JWM, et al. Low plasma selenium concentrations, high plasma human immunodeficiency virus load and high interleukin-6 concentrations are risk factors associated with anemia in adults presenting with pulmonary tuberculosis in Zomba district, Malawi. *Eur J Clin Nutr*. 2005;59(4):526–532.

100. Harms TF. Summary statistics for selenium in vegetation calculated from U.S. Geological Survey data; 1995. 40 p.

101. Xin PL, Hai YW, Zhang C, et al. Research on Active Selenium Polysaccharide in Artemisia Argyi. *Advanced Materials Research*. 2013;634-638:1054–1057.

102. El Mehdawi AF, Quinn CF, Pilon-Smits E. Selenium Hyperaccumulators Facilitate Selenium-Tolerant Neighbors via Phytoenrichment and Reduced Herbivory. *Curr Biol*. 2011;21(17):1440–1449.

103. Jyoti MA, Nam KW, Jang WS, et al. Antimycobacterial activity of methanolic plant extract of Artemisia capillaris containing ursolic acid and hydroquinone against Mycobacterium tuberculosis. *J Infect Chemother*. 2016;22(4):200–208.

104. Jaki BU, Franzblau SG, Chadwick LR, et al. Purity-activity relationships of natural products: the case of anti-TB active ursolic acid. *J Nat Prod*. 2008;71(10):1742–1748.

105. Qiu F, Cai G, Jaki BU, et al. Quantitative purity- activity relationships of natural products: the case of anti-tuberculosis active triterpenes from Oplopanax horridus. *J Nat Prod*. 2013;76(3):413–419.

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