



Action of *Artemisia annua* on adaptive immunity in COVID-19 infections

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Abstract

Antiviral herbal medicines have already been used in many epidemics, notably in the two previous coronavirus outbreaks - MERS-CoV in 2012, SARS-CoV in 2013 - or in seasonal epidemics caused by influenza or dengue viruses.

In coronavirus infection (COVID-19), cellular adaptive immunity is primarily involved, in particular CD8 and CD4 lymphocytes that stimulate the B lymphocytes responsible for the production of antibodies targeting the coronavirus. In addition, there is a cytokine storm in patients infected with COVID-19 responsible for a major inflammatory response and their very severe progressive clinical state. The increase in interleukin-10 and TNF alpha reduces CD4 counts, causes functional exhaustion of immune cells and induces, at their site of action (liver, vascular endothelium), runaway production and action of inflammation proteins, causing the secondary aggravation of COVID-19 patients.

Artemisia annua has a recognized antiviral activity (anti HSV1, Poliovirus, RSV, hepatitis C anti-virus, type 2 dengue virus, hantavirus) and anti HIV in vitro thanks to the flavonoids, quercetin and dicaffeoylquinic acids it contains. These molecules have been shown to inhibit the enzymatic activity of MERS-CoV-3 CLPro (MERS-CoV-3 chymotrypsin-like protease), an enzyme also produced by SARS-CoV-2.

The antiviral action of *Artemisia annua*, which is achieved by stimulating adaptive immunity, regulating the production of the pro-inflammatory cytokines prostaglandin E2 (PGE2), IL-6, IL-10, TNF alpha and increasing the genesis of CD4, CD8 and interferon gamma, involves many minerals and biomolecules: the properties of flavonoids, polyphenols, triterpenes, sterols, saponins, polysaccharides, artemisinin and its derivatives, the concentration of zinc, gallium and selenium in the plant play a role in the immune, antiviral, antioxidant and anti-inflammatory response.

The plant is extremely rich in Vitamins A and E, of which one, when supplemented, is known to reduce morbidity and mortality in viral infections, HIV among others, and the other is a powerful antioxidant.

It is therefore the combination of these biomolecules and the intake of *Artemisia annua* in totum that could improve exhausted adaptive immunity and modulate the runaway inflammatory response during COVID-19 infection, as this plant has already demonstrated in other serious viral and parasitic infections.

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In 2007, during the SARS epidemic, several randomized controlled studies were conducted to evaluate the effectiveness of combining traditional Chinese and western medicine compared to western medicine alone.

A meta-analysis of these studies was conducted using recent data from the literature validating the therapeutic effects of traditional Chinese medicine combined with western medicine, in order to select the studies using the validated treatments combining these two types of medicine.

24 studies conducted between 2002 and 2006 were included in the meta-analysis. Among the criteria studied, the disappearance of the pulmonary infiltrate was significantly faster in the group combining the two types of medicine. Ten of the 24 studies used corticosteroids, the average daily dose of corticosteroids used was significantly lower in the group combining the two types of medicine compared to the group using only western medicine. In 4 studies, CD4 levels were measured at the start and end of treatment. There was a significant difference in favour of the combination of the two types of medicine in the increase in CD4 levels at the end of treatment.

Yan Chen, Jeff J. Guo, Daniel P Healy and Siyan Zhan

Effect of integrated traditional Chinese herbal medicine and western medicine on the treatment of severe acute respiratory syndrome

A meta-analysis

Pharmacy Practice 2007; 5 (1):1-9

I. COVID-19 immune study

COVID-19 is a single-stranded, positive RNA virus.

Sequence analysis shows that COVID-19 has a genome typical of the coronavirus structure and belongs to a group of β -coronaviruses that include SARS-CoV-1 and MERS-CoV.

The COVID-19 genome is more than 85% identical to SARS-CoV-1.

During infection with COVID-19 innate immunity is little used (phagocytosis by macrophages and polymorphonuclear neutrophils). The polymorphonuclear neutrophil count is little or not increased in clinical studies except at the end of the ICU stay, in the late stage.

In this infection, the body's immune response is mainly based on adaptive defence mechanisms :

- B lymphocytes stimulated by CD4 lymphocytes are responsible for the production of specific antibodies targeting SARS-CoV-2, which peak at day 8 (onset of IGG production) and are present in the blood until day 20 after infection; IGMs only seem to appear from day 9 after infection and last for three weeks. There is still little data on this kinetics.

- The so-called "Killer T" lymphocytes, which can directly destroy foreign particles.

The other population of activated lymphocytes is made up of CD4^+ follicular helper lymphocytes, specific to the germinal centres (lymph node and spleen). They are located in the secondary lymphoid organs and participate in the T-cell-dependent humoral response.

T4 and T8 lymphocytes (CD4 and CD8): During SARS-CoV-2 infection, CD4 and CD8 levels and the CD4/CD8 ratio are significantly lowered, low CD4 levels could be a predictor of patient survival, especially in subjects over 60 years of age.

CD4 cells express molecular signs of functional exhaustion in very severe patients. There is major secretion of interleukin-6 (IL-6), interleukin-10 (IL-10) and TNF alpha, particularly in patients admitted to ICU who are experiencing this cytokine shock.

Interleukin-10 is very high in COVID-19 infection, inhibits cell proliferation and may induce CD4 cell exhaustion, as in HIV infection.

Interleukin-6 levels are also very high. IL-6 is a cytokine of the acute phase of inflammation. It is ubiquitous. It induces the hepatic production of inflammation proteins.

Finally, TNF alpha is also at a high level: it is a pro-inflammatory cytokine that acts on the vascular endothelium.

Numerous studies also report the appearance of diffuse microthromboses and signs of disseminated intravascular coagulation (DIC) in the severe stage of the disease, associated with thrombopenia.

N Eng J M 2020. Zhu N, Zhang D, Wang W, et al.
A novel coronavirus from patients with pneumonia in China, 2019
 The New England Journal of Medicine

Chen N, Zhou M, Dong X et al
Epidemiological features of patients infected with 2019 novel coronavirus in Wuhan, China
 Lancet 2020

Bo Diao, Chenhui Wang, Yingjun Tan, Xiewan Chen.
Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19)

Preprint DOI <https://doi.org/10.1101/2020.02.18.20024364>.

II. *Artemisia annua* biomolecules interacting with activated immunity in COVID-19 infection

The various tissues of *Artemisia annua* contain hundreds of phytochemical compounds that are well below recommended toxicity levels.

Many of these compounds have antioxidant, antiparasitic, antibacterial and antiviral properties:

Duke JA (2001)

Handbook of phytochemical constituents of GRAS herbs and other economic plants

CRC Press LLC, Boca Raton, FL p 70

Ebiamadon Andi Brisibe, Umoren E. Umoren, Fraideh Brisibe, Pedro M.

Magalhaës et al.

Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L.

Food Chemistry.115 (2009) 1240 -1246.

II.1 Antiviral activity

The antiviral action of *Artemisia annua* is carried out by stimulating adaptive immunity through the regulation of the production of pro-inflammatory cytokines IL-6, IL-10, TNF alpha and the stimulation of the genesis of CD4 and CD8 and interferon gamma.

Artemisia annua dry extracts have an inhibitory effect on the secretion of nitrogen monoxide (NO) induced by lipopolysaccharides and on the production of prostaglandin E2 (PGE2).

Artemisinin, in dry extracts of *Artemisia annua* taken as an herbal tea, has better bioavailability, crosses the intestinal wall better and is three times more soluble than pure artemisinin. Due to the inhibition of CYP2B6 and CYP3A4 by the other molecules present in the herbal tea, artemisinin in the dry extracts regulates the production of pro-inflammatory cytokines more effectively than pure artemisinin.

Matthew R, Desrosier, Alexis Mittleman and Pamela Weathers

Dried Leaf *Artemisia annua* Improves Bioavailability of Artemisinin via Cytochrome P450 Inhibition and Enhances

Artemisinin Efficacy Downstream

Biomolecules, 2020, 10, 254, doi : 10 .3390.

Kim WS, Choi WJ, Lee S, Kim WJ, Lee DC, Sohn UD, Shin HS, Kim W
**Anti-inflammatory, Antioxidant and Antimicrobial Effects of
Artemisinin Extracts from *Artemisia annua* L.**

Korean Journal of Physiology & Pharmacology. 2015 Jan;19(1):21-7.

An in vitro study comparing the antiviral activities of 7 species of *Artemisia* (HSV1, para-influenza virus, poliovirus 1 and respiratory syncytial virus) showed that the antiviral activity of *Artemisia annua* was the strongest and, in some cases, superior to that of Acyclovir. It is the quercetins that induce a decrease in viral activity and concentration.

Mehrangiz Khajeh Karamodini, Seyed Ahmad Emami et al.

**Antiviral activities of aerial subsets of *Artemisia* species against
Herpes Simplex virus type 1 (HSV1) in vitro**

Asian Biomedicine, vol. 5, Issue 1, 2011 pp. 63-68

An in vitro study has demonstrated the powerful anti HIV activity of *Artemisia annua* herbal tea. This activity is not due to artemisinin: it is the dicaffeoylquinic acids (dicaffeoylquinic acids) that are active. No cellular toxicity was found.

Andrea Lubbe, Isabel Seibert, Thomas Klimkait, Frank van der Kooy

**Ethnopharmacology in overdrive: the remarkable anti-HIV
activity of *Artemisia annua***

Journal of Ethnopharmacology (2012) Jun 14;141(3):854-9.

Flavonoids

Artemisia annua contains chalcones and quercetin-3-beta-d glycosides (quercetin-3- β -d-glucoside). These are molecules which, in addition to their antioxidant role, have recognised antiviral properties. These natural molecules have inhibitory activity on 3C-like protease (3CLprot). They are also inhibitors of the chymotrypsin-like protease produced by SARS-COV2.

Jorge F.S. Ferreira, Devanand L. Luthria, Tomikazu Sasaki, and Arne Heyerick

Flavonoids from *Artemisia annua* L. as antioxidants and their

potential synergism with artemisinin against malaria and cancer

Molecules. 2010 May; 15(5): 3135–3170.

Jo S, Kim S, Shin DH, Kim MS

Inhibition of SARS-CoV 3CL protease by flavonoids

J Enzyme Inhib Med Chem. 2020; 35; 145 – 151.

Seri Jo, Hyo-Jin Kim, Suwon Kim, Dong Hae Shin, Mi-Sun Kim

Characteristics of flavonoids as potent MERS-CoV 3C-like protease inhibitors

Chemical Biology & Drug Design (2019); 19 : 2023-2030

Quercetin found in aqueous extracts of *Artemisia tschneiviana*, but which is also present in *Artemisia annua*, has a powerful antihemolytic action.

Naqinezhad A, Nabavi SM, Nabavi SF, Ebrahimzadeh MA.

Antioxidant and antihemolytic activities of flavonoid rich fractions of *Artemisia tschneiviana* Besser

European Review for Medical and Pharmacological Sciences,
2021 Jul; 16 Suppl 3: 88-94.

Flavonoids, casticin, and chrysopterin 6-D reduce inflammation in vitro and in vivo.

Li YJ, Guo, Yang Q, Weng XG, Yang L, Wang YJ, Chen Y, Zhang D, Li Q, Liu XC,
Kan XX, Chen X, Zhu XX, Kmoníèková E, Zídek Z

Flavonoids casticin and chrysopterin D from *Artemisia annua* L. inhibit inflammation in vitro and in vivo

Toxicology and Applied Pharmacology, 17 Apr 2015, 286(3): 151-158

Quercetins belong to the group of bioflavonoids, they have antiviral activities against HSV1, HSV2, cytomegalovirus and some types of adenovirus.

In the following study, quercetins express significant in vitro anti-viral activity (on replication) against RNA dengue virus type 2:

Keivan Zandi, Boon Teong Teoh, Sing – Sin Sam,
Pool, Fong Wong, Moh Rais Mustafa and Sazaly Abubakar
**Antiviral activity of four types of bioflavonoid
against dengue virus type-2**
Virology Journal 2011, 8, 560

Dicafeylquinic acid / chlorogenic acid

Artemisias and *Artemisia annua* are rich in chlorogenic and other caffeoylquinic acids which are used in the treatment of viral hepatitis and other viruses.

Zhao Wen-wen, Zhang Wei-na, Chen Yu-ru, Yang Fengping, Cao Qiming, Chen
Wen-zhong, Liu Jun-li, Dai Kewei
**Identification and purification of novel chlorogenic acids
in *Artemisia annua* L.**
Journal of Experimental Biology and Agricultural Sciences
October - 2015; Volume – 3(V) ISSN 2320 – 8694.

Yi-hang Wu, Bing-jie Hao, Hong-cui Cao, Wei Xu, Yongjun Li, Lanjuan Li
**Anti-Hepatitis B Virus Effect and Possible Mechanism of Action of
3,4-O-Dicaffeoylquinic Acid In Vitro and In Vivo**
Evidence-based Complementary and Alternative Medicine
June 2012 (1, supplement): 356806

Xueyun Zheng, Ryan S. Renslow, Mpho M. Makola, Ian K. Webb, Liulin Deng &
al,
**Structural Elucidation of *cis/trans* Dicaffeoylquinic Acid
Photoisomerization Using Ion Mobility Spectrometry-Mass
Spectrometry**
The Journal of Physical Chemistry Letters, 2017, 8, 1381-1388.

Mpho M. Makola, Ian A. Dubery, Gerrit Koorsen, Paul A. Steenkamp, Mwadham
M. Kabanda, Louis L. du Preez, and Ntakadzeni E. Madala
**The Effect of Geometrical Isomerism of 3,5-Dicaffeoylquinic Acid
on Its Binding Affinity to HIV-Integrase Enzyme:
A Molecular Docking Study**
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 4138263

Sterols

Known for years by the Chinese, the sterols of *Artemisia annua* have viral inhibition properties superior to other molecules of the plant such as artemisinin or arteannuin B.

Its antiviral activity stands out from that of 20 medicinal plants studied. Sterols interfere with the synthesis of the cholesterol envelope of the influenza virus and markedly reduce its aggressiveness.

Xiangjie Sun and Gary Wittaker

Role of Influenza Virus Envelope Cholesterol in Virus Entry and Infection

Journal of Virology, 2003 Dec; 77(23): 12543–12551.

Interleukin-6 (IL-6) is high in HIV infections. Products containing phytosterols lower the concentration of interleukin-6.

Breen EC, Rezai AR, Nakajima K, Beall GN, Martinez-Maza O

Infection with HIV is associated with elevated IL-6 levels and production

Journal of Immunology, 1990 Jan 1 (V. 1444 (2) : 480 6 484.

Among 21 medicinal plants evaluated, extracts of *Artemisia annua* have the greatest antiviral inhibitory activity against tobamovirus. Sterols such as sitosterol and stigmasterol are responsible for this inhibitory activity in *Artemisia annua*.

MM Abid Ali Khan, DC Jain, RS. Bhakuni, Mohd. Zaim, RS Thaku.

Occurrence of some antiviral sterols in *Artemisia annua*

Plant Science. Volume 75 , Issue 2, 1991, Pages 161-165 .

Beta-sitosterols et Beta-sitosterol glucosides

The antiviral β -sitosterols and the β -sitosterol glucosides are also present in *Artemisia annua*. They stimulate the cellular immune response that controls viral replication.

This antiviral activity has been shown in HIV-infected patients. They stabilize the CD4 level of infected individuals and reduce the production of interleukin-6.

Patric Jacques Desire Bouic
**Use of a combination of beta-sitosterol and beta-sitosterol glucoside
for treating HIV infection**
European Patent Office 0858806A1.

Artemisia annua increases the number of activated D lymphocytes.

Constant Kansango Tchandema, Pierre Lutgen
**In vivo trials on the therapeutics effects of encapsulated
Artemisia annua and *Artemisia afra***
Global Journal For Research Analysis, Volume V, Issue VI, June 2016

Aqueous extracts of *Artemisia annua* stems and leaves lead to a proliferation of T, CD4 and CD8 lymphocytes.

Mohamed Islamuddin, Garima Chouhan Abdullah Farooque
**Th1-Biased Immunomodulation and therapeutic potential of
Artemisia annua in Murine Visceral Leishmaniasis**
PLOS Neglected Tropical Diseases. January 8, 2015 9

Dihydroartemisinin or dihydro-qinghaosu - DHA or DQHS

Extracts of *Artemisia annua* and its dihydroartemisinin (DHA) derivatives and artemether and artesunate are used for the treatment of malaria and many other acute or chronic parasitoses in which the inflammatory reaction of the body is sometimes inadequate, too great, poorly regulated.

Artemisia annua herbal tea is thus used for the treatment of malaria:

Munyangi J, Cornet-Vernet L, Idumbo M, Lu C, Lutgen P, Perronne C, Ngombe N, Bianga J, Mupenda B, Lalukala P, Mergeai G, Mumba D, Towler M, Weathers.
***Artemisia annua* and *Artemisia afra* tea infusions vs. artesunate-amodiaquine (ASAQ) in treating *Plasmodium falciparum* malaria in a large scale, double blind, randomized clinical trial**
Phytomedicine, 2019, 57, 49- 56.

Dihydroartemisinin (DHA) is used in chronic arthritis, indicating that it is involved in rebalancing the immune response in individuals with impaired immunity.

In healthy mice infected with *Toxoplasma gondii* and *Plasmodium berghei* and then treated with DHA, the study of the immune response shows an increase in the

splenic index (enlargement of the spleen) caused by the genesis of CD4 lymphocytes and additional CD8 lymphocytes in the spleen and circulation. DHA increases the proportion of T helper cells and CD8n cells and decreases the number of circulating and splenic B cells. DHA may reduce the production of pro-inflammatory cytokines. In addition to its antiparasitic actions, DHA modulates the immune response of the infected host.

Zhang T, Zhang Y, Jiang N, Zhao X, Sang X, Yang N, Feng Y, Chen R, Chen Q
Dihydroartemisinin regulates the immune system by promotion of CD8⁺T lymphocytes and suppression of B cell responses
Science China Life Sciences 2019 July 8.

The levels of anti-dsDNA antibodies and TNF alpha were lower in the group of BXSB mice with lupus nephritis receiving high and moderate doses of dihydroartemisinin (from dihydro-qinghaosu) than in the control group.

Yan-jun Dong, Wei-dong Li, You-you Tu
Effect of dihydro-qinghaosu on auto-antibody production, TNF alpha secretion and pathologic change of lupus nephritis in BXSB mice
Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi (Chinese journal of integrated traditional and Western medicine) 2003 Sep;23 (9): 676-9.

Matt Desrosier, Pamela J. Weathers
Effects of leaf digestion and artemisinin solubility for oral consumption of dried *Artemisia annua* leaves to treat Malaria
Journal of Ethnopharmacology, 2016, 190, 313-318.

II.2 Oxidative/anti-oxidative capacity of *Artemisia annua* plant and role in immunomodulation

Artemisia annua has both oxidative and non-oxidative properties that intervene in a balanced way at different stages against pathogenic microorganisms infesting the host.

Our bodies are constantly producing hydrogen peroxide. White blood cell peroxidases block organic molecules and generate H₂O₂ as a by-product. Hydrogen peroxide participates in the elimination of viruses and bacteria.

H₂O₂ is generated and destroyed by specific enzymes which suggests that the intracellular H₂O₂ concentration is carefully regulated.

Superoxide dismutase catalyzes the dismutation of the superoxide radical into H₂O₂ and an oxygen molecule.

Arginine, which is abundant in *Artemisia annua*, can also generate H₂O₂ via antioxidant iNO synthase enzymes.

H₂O₂ can react with iron or copper to produce highly reactive OH radicals.

In the liver, in addition to its cytotoxic effects, H₂O₂ plays an essential role as a molecule regulating the activation of the cellular immune defence.

The natural support of H₂O₂ in the organism attacked by a virus may be exhausted, an H₂O₂ supply from a medicinal plant in this context could be beneficial and improve the cellular immune response.

Molecules such as artemisinic acid and arteannuin B also play a role in oxidative stress. A study analysing the concentration of these compounds in Chinese *Artemisia annua* (stems) showed that they generate a high level of H₂O₂ in the PPVPP test.

Ludjain AL. Sowaimel compared the amount of H₂O₂ in Chinese and Yugoslavian *Artemisia annua*:

Ludjain AL Sowaimel

**Artemisinin yields in Chinese and Yugoslavian strains
of *Artemisia annua***

Project Report submitted to the Faculty of the Worcester Polytechnic Institute in
partial fulfilment of the requirements for the Degree of Bachelor of Science,
Worcester Polytechnic Institute April 30, 2009

H₂O₂ activates lymphocytes:

Los M, Dröge W, Stricker K, Baeuerle PA, Schulze- Osthoff K

Hydrogen peroxide as a potent activator of T lymphocyte functions.

European Journal of Immunology. 1995 Jan ; 25 (1) : 1596 165.

H₂O₂ also acts as an intracellular messenger in activated lymphocytes:

Reth M

Hydrogen peroxide as second messenger in lymphocyte activation.

Nat Immunol. 2002 Dec ; 3 (12): 1129 – 34.

Cytochrome P450 enzymes have distinct activity in the production of hydrogen peroxide and large amounts are present in liver microsomes.

Among these enzymes, the most active are CYP1A1 and CYP3A4. *Artemisia annua* is an inducer of CYP3A4 leading to the acceleration of its own metabolism and that of other xenobiotics.

Vladimir Mishin Diane E.Heck , Debra L laskin and Jeffrey D Laskin
Human recombinant cytochrome P450 enzymes display distinct hydrogen peroxide generating activities during substrate independent NADPH oxidase reactions
Toxicological Sciences. 2014 Oct; 141(2): 344-52

Scopoletin

Scopoletin found in large quantities in *Artemisia annua* is a powerful antioxidant and a scavenger of hydrogen peroxide.

Scopoletin, in addition to its major antioxidant property, has an anti-inflammatory effect via its prostaglandin E2 (PGE2) inhibitory activity and strongly inhibits the production of iNO synthase.

Artemisia annua is also rich in vitamin E and zinc and potassium, three other major antioxidants.

Sugunya Utaida, Saranya Auparakkitanonand Prapon Willairat
Synergism and antimalarial antibiotics with hydrogen peroxide in inhibiting Plasmodium falciparum growth in culture.
Southeast Asian J Prop Med Public Health, 2014, 45, 165.

X Yao, Z Ding, Y Xia, Y Dai
Inhibition of monosodium urate crystal-induced inflammation by Scopoletin and underlying mechanisms
Internal Immunopharmacology, 2012, 14, 454-462.

Polyphenols

Artemisia annua is rich in polyphenols which generate hydrogen peroxides. In addition, polyphenols are known for their platelet anti-aggregation activity. They may inhibit microthrombosis formation and stabilize platelet levels during severe disease progression.

Iqbal Hussain, Farhat ali Khan, Muneeb Ur,
Muhammad Muneeb ur Rehman Khattak

Evaluation of Inorganic Profile of Selected Medicinal Plants of Khyber Pakhtunkhwa Pakistan

World Applied Sciences Journal 12(9):1464-1468 · September 2011

Christian Neu

Les interactions entre les antithrombotiques et les plantes médicinales

Sciences pharmaceutiques 2011

HAL 01731807

Polysaccharides

In mice, polysaccharides, extracted from *Artemisia annua*, used as adjuvants for the hepatitis C vaccine, increase the secretion of interferon gamma and interleukin-4. Interferon gamma is a Th1 cytokine and interleukin-4 is a Th2 cytokine. The secretion of IFN gamma is higher than that of interleukin-4, suggesting that in this case *Artemisia annua* is more effective in inducing a cellular immune response.

However, the secretion of IFN gamma could also directly facilitate B cell differentiation and, in the meantime, stimulate their antibody secretion and thus potentiate the humoral immune response.

Bao LD, Ren XH, Ma RL, Wang Y, Yuan HW, Lv HJ

Efficacy of *Artemisia annua* polysaccharides as an adjuvant to hepatitis C vaccination

Genetics and molecular research 1' (2): 4957- 4965 (2015)

Significantly increasing CD4 and CD8 levels, polysaccharides stimulate the immune anti-tumor defense. A secondary increase in IFN Gamma and interleukin-4 appears.

Thus the polysaccharide extracts from *Artemisia annua* highlight, in this mouse animal model carrying hepatic carcinoma, the different immune modulation pathways activated by these molecules.

Jiayu Chen, Jiaqi Chen, Xintai Wang, and Chibo Liu
**Anti-Tumour Effects of Polysaccharides Isolated from
Artemisia Annua L. by Inducing Cell Apoptosis and
Immunomodulatory Anti-Hepatoma Effects of Polysaccharides**
African Journal of Traditional, Complementary and Alternative medicine
(2014) 11 (1): 15-22.

The microbiota of the infested host is also involved in the body's defences and its composition could be a risk factor in serious viral infections as is the case in *Plasmodium falciparum* parasitic infections.

Among the polysaccharides present in *Artemisia annua* is inulin, in significant quantities. In addition to its role in the direct stimulation of B lymphocytes, it is an indigestible prebiotic which enhances the probiotic effect of microorganisms such as *Lactobacillus rhamnosus* or *Bifidobacterium lactis* that balance the microbiota of the infected host.

Villarino NF, Gary R, Le cleir, Joshua E, Denny, Sarah S et al.
Composition of the gut microbiota modulates the severity of Malaria
Proc Natl AcadSci U.S.A. 2016, 13; 113(8): 2235-40.

Monika Roller, Gerhard Rechkemmer, Bernhard Watzl
**Prébiotic Inulin Enriched with oligofructose in combination with the
probiotics *Lactobacillus Rhamnosus* and *Bifidobacterium lactis*
modulates intestinal immune function in rats**
Nutritional Immunology- Research Communication 2004, 153-156.

Gibson GR, Beatty ER, Wang X, Cummings JH
**Selective stimulation of bifidobacteria in a human colon
by oligofructose and inulin**
Gastroenterology. 1995 Apr ; 108(4): 975-82.

Triterpenes

The triterpenes in the plant interfere with platelets and have platelet anti-aggregating activity.

Artemisia annua plant extracts increase and stabilize platelet levels during malaria and increase patient survival. The pentacyclic triterpenes present in the plant are involved in this phenomenon. In a study conducted in Brazil, patients treated with *Artemisia annua* herbal tea had shorter bleeding times than those treated with Coartem (artemisinin). The latter had lower platelet levels than those in the group treated with *Artemisia annua* herbal tea..

Pierre Lutgen & Jérôme Munyangi

Platelets, eryptosis, amiodarone aspirin, Artemisia

Pharmacy and pharmacology International Journal 2018 (6) : 377-381

Christian Neu

Les interactions entre les antithrombotiques et les plantes médicinales

Sciences pharmaceutiques 2011, HAL 01731807

II.3 Studies of biomolecules : minerals contained in the plant.

Selenium

Selenium, available in large quantities in the plant, participates in the regulation of cytokines (down-regulation of interleukin-8) and the increase of CD4 in acute or chronic viral infections.

Plants of the *Artemisia* family accumulate many minerals including selenium. Selenium concentrations are ten times higher than those of other fruits and vegetables.

Thelma F. Harms

Summary statistics for selenium in vegetation

from U.S. Geological Survey data

1999 Bulletin 2117.

Selenium is found in significant amounts in immune tissues such as the liver, spleen and lymph nodes.

Decreased CD4 count is a marker for human immunodeficiency syndrome. Retroviruses such as HIV lower the selenium level of their host and reduce the level

of glutathione. This leads to a reduction in the CD4 count. In mice supplemented with selenium for 8 weeks, there is an increase in CD4 counts that is proportional to the dose of selenium received in their diet.

Selenium supplementation regulates the production of interleukin-2 leading to proliferation of NK lymphocyte cells. Selenium supplementation down-regulates the hyper production of interleukin-8 present during inflammation.

Baum MK. Miguez – Burbano MJ. Campa A. Shor – Posner G
**Selenium and Interleukins in Persons Infected with Human
Immunodeficiency Virus Type 1**

Journal of Infectious Diseases, 2000 Sep; 182 Supplem

The role of selenium in the evolution of viral pathologies and their progression or not according to its concentration is described in:

Paweł Zagrodzki

Selenium and the Immune System

Postępy Higieny i Medycyny Doświadczalnej

(Advances in Hygiene and Experimental Medicine) 58:140-9 · April 2004

John R. Arthur, Roderick C. McKenzie, Geoffrey J. Beckett

Immunity Enhanced by Trace Elements :

Selenium in the Immune System

The Journal of Nutrition, Volume 133, Issue 5, May 2003, Pages 1457S–1459S

Peter R. Hoffmann and Marla J. Berry

The influence of selenium on immune responses

Mol Nutr Food Res. 2008 Nov; 52(11): 1273–1280.

In China, hantavirus hemorrhagic fevers are associated with selenium deficiency. The intake of selenium leads to a decrease in viral replication.

Li- QunFang, Marco Goeijenbier, Shu-Quing Zuo,

**The Association between Hantavirus Infection and Selenium Deficiency
in Mainland China**

Virus, 2015, 7, 333-351.

Gallium

Available in large quantities in the plant, gallium participates in the regulation of cytokines: down-regulation of interleukin-6 and TNF alpha and inflammation cytokines.

Gallium accumulates in inflammatory lesions. The permeability index of inflammatory tissues is higher than in healthy tissues. Gallium remains in these tissues by binding to the mucopolysaccharide acid also present in these inflammatory tissues. Overproduction of TNF is one of the major mechanisms responsible for the development of fever in infections. Gallium nitrates inhibit the production of inflammation mediators such as interleukin-6, TNF alpha.

Min-fu Tsan,

Mechanism of Gallium – 67 Accumulation in Inflammatory Lesions

The Journal of Nuclear Medicine, 26, 88-92. 1985.

Gallium nitrates are very effective in reducing or even eliminating joint pain.

G Eby

Elimination of arthritis pain and inflammation for over 2 years with a single 90 minutes, topical 14% gallium nitrates treatment : case reports and review of actions of gallium III

Med. Hypotheses, 2005, 65 11”–15.

Gallium nitrates are also prescribed in lupus erythematosus and chronic joint pain.

Zinc

Artemisia annua is one of the three plants richest in zinc. It is considered a super accumulator of zinc. Zinc is involved in the entire immune system.

It has a pivotal role in host resistance to viral, fungal and bacterial infections. Zinc also functions as an anti-oxidant. It protects the cell against damage from oxygen free radicals generated during immune activation.

Humans have no zinc reserves, therefore a daily intake of zinc is necessary and supplementation helps to overcome serious viral infections. A deficiency or lack of zinc intake severely damages the immune system, especially with regard to the

adaptive defence of T-lymphocytes. This leads to a reduction in the number of CD4 cells and T cells, a decrease in the ratio of type 1 and type 2 helper T cells with a decrease in the production of type 1 helper T cells and an alteration of the immune defence derived from these cells.

Disturbances in zinc homeostasis lead to an increased risk of infection and zinc supplementation restores immune function. The evolution of viral pathologies can thus be influenced by zinc supplements. Type 1 T helper lymphocytes stimulate macrophages that phagocyte the attacking pathogens.

A high CD4/CD8 ratio indicates a better immune activity. Zinc thus stimulates the immune system by increasing the CD4 count.

Hönscheid A, Rink L, Haase H.

**T- lymphocytes : a target for stimulatory
and inhibitory effects of zinc ions**

Endocrine, Metabolic & Immune Disorders - Drug Targets
2009 Jun ; 9(2) : 132-44 Review.

A study conducted in Mexico City on pulmonary tuberculosis showed that the more rapid disappearance of bacillus from sputum was associated with an improvement in zinc intake, which was linked to a better response of type 1 helper lymphocytes.

In this clinical study, this adjuvant therapy shortened the contagion time of tuberculosis patients.

Armijos RXI, Weigel MM, Chacon R, Flores L, Campos A,

Adjunctive micronutrient supplementation for pulmonary tuberculosis

Salud Publica Mex 2010 May – June ; 52 (3) : 185 – 189.

Zinc supplementation improves the cellular immune response by increasing CD4 and CD4/CD8 ratio as found in a randomized study in 76 children in India.

Such supplementation could have an impact on diarrhoeal morbidity in children.

Sazawal S. Bentley M. Black RE. Dhinga P, Georges S, Bhan MK,

**Effect of zinc supplementation on observed activity in low
socioeconomic Indian preschool children**

Pediatrics, 1996 Dec; 98 (6 Pt 1) : 1132-7.

Coronavirus papain-like protease (I PLP) is a novel deubiquitinase. It is an interferon antagonist and thus inhibits the host's innate immune response. High doses of Zinc inhibit this protease.

Manisha Prajapa , Phulen Sarma and Bikash Medhi

Drug Targets for coronavirus: A systematic review

Indian Journal of Pharmacology , 2020 DOI; 10.4103/ijp.IJP_115 120

Baez-Santos YM, Barraza SJ, Wilson MW, Agius MP, Mielech AM, Davis NM et al
X-ray structural and biological evaluation of a series of potent and highly selective inhibitors of human coronavirus papain-like proteases

Journal of Medicinal Chemistry. 2014; 57: 2393 – 2412

Gosh Ak, Takayama J, Rao KV, Ratia K, Chaudhuri R, Mulhearn DC, et al,
Severe acute respiratory syndrome coronavirus papain-like novel protease inhibitors : design, synthesis, protein-ligand x-ray structure and biological evaluation

Journal of Medicinal Chemistry. 2010; 53 : 4968-79

Zinc and amino acids

Artemisia annua is rich in zinc and arginine. Arginine easily forms a zinc-arginine complex in a wide range of concentrations.

Emilio Bottari, Maria Rosa Festa, Lorella Gentile

An Investigation on the Equilibria between Arginine and Iron(II) and Iron(III)

Journal of Medicinal Chemistry. Eng. Data Feb.2013, 58, 3, 718-723

Arginine is present in large quantities in *Artemisia annua*, along with most of the essential amino acids. Arginine produces NO thanks to iNO synthase. It improves and participates in the regulation of the body's innate immune defences.

Virginie Mieulet et Richard F.Lamb

**Arginine et réponse immunitaire innée:
Au-delà de la production de monoxyde d'azote**

Med Sci (Paris) 2011 ; 27 :461-463

Proline down-regulates CD4 counts in viral infections. Many viruses have proline-rich domains. (Herpes, hepatitis, influenza viruses).

Zinc plays a key role in amino acid metabolism. It binds to the enzyme inhibiting nitric oxide synthase (iNOS) and inhibits the production of NO.

Zinc inhibits the transport of proline to cells. It interacts with other amino acids:

Cortese- Krott MM, KulatkovL, Oplander C.

**Zinc regulates iNOS-derived nitric oxide formation
in endothelial cells.**

Redox Biol. 2014 JUL 16 ; 2: 945-54 . Doi : 10.1016 / j.Redox . 2014.0601.

Potassium et saponins

The concentration of potassium is very high in *Artemisia annua* which could explain its antioxidant properties. *Artemisia annua* contains only traces of sodium.

Saponins stimulate the bioavailability of the plant and activate the cell dependent calcium channels.

I.Hussain

**Evaluation of inorganic profile of selected medicinal
plants of Khyber Pakhtunkhwa Pakistan**

World Appl SC J 12(9) / 1464-1468

H.Oberleithner, C.Callier, H.E. de Wardener

**Potassium softens vascular endothelium
and increases nitric oxide release.**

PNAS 2009 106,8, 2829-2834

Mc Manus OB, Harris GH, Gianegiacomo KM

**An activator of calcium-dependant potassium channels isolated from
medicinal herb**

Biochemistry, 1993 Jun 22; 32(24):6128-33

Adjuvants using saponins stimulate cellular immunity to improve antibody production, even in small doses:

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

III. Conclusion

In coronavirus infection (COVID-19), cellular adaptive immunity is primarily involved, in particular CD8 and CD4 lymphocytes that stimulate B lymphocytes responsible for the production of antibodies targeting coronavirus.

The CD4 /CD8 level is collapsed in infected patients and the lowering of the CD4 level may be a predictor of severity in the evolution of the disease.

In addition, there is a cytokine storm in these patients, responsible for the runaway inflammatory response and major secondary clinical aggravation. The increase in interleukin-10 and TNF alpha reduces CD4 counts and causes functional exhaustion of immune cells, triggering, at their site of action (liver, vascular endothelium), a runaway production and action of the inflammation proteins responsible for the secondary aggravation in COVID-19 patients.

Artemisia annua has known antiparasitic activity but also antiviral activity (anti HSV-1, poliovirus, RSV, hepatitis C anti-virus, dengue virus type 2 , hantavirus) and anti HIV in vitro thanks to the flavonoids, quercetin and dicaffeoylicinic acids it contains.

These molecules have been shown to inhibit the enzymatic activity of MERS-CoV-3 CLPro (MERS-CoV-3 chymotrypsin-like protease), an enzyme also produced by SARS-CoV-2.

Sterols, very numerous in the plant, interfere with synthesis of the viral membrane. In addition, the saponins of the plant stimulate the bioavailability of artemisinin, cellular immunity and improve the production of antibodies. The polyphenols contained in *Artemisia annua* generate hydrogen peroxide, a super-oxidant, but the plant also contains scopoletin, vitamin E and other super-oxidants that intervene at different stages to fight the pathogen and strengthen the host's defences.

The polysaccharides of *Artemisia annua* increase the secretion of interferon gamma and interleukin-4 which act by increasing CD4 and CD8 levels. Dihydroartemisinin decreases the production of pro-inflammatory cytokines and increases the amount of CD8 T lymphocytes. It is also a powerful antioxidant.

Triterpenes and polyphenols present in large quantities are platelet antiaggregants, which could be of interest in patients to prevent the appearance of microthromboses described in the evolution of COVID-19.

The concentration of zinc in the plant is significant, *Artemisia annua* is an accumulator of this mineral. Humans have no reserves of zinc. In periods of viral infection, zinc intake is essential to stimulate the adaptive immune defences, those which are exhausted by the coronavirus. Zinc increases the CD4 level and the type 1 T

helper cells. At high doses, it inhibits a SARS CoV-2 enzyme, papain-like protease, and improves the production of interferon alpha involved in innate immunity. Zinc also functions as an antioxidant. It protects the cell against damage from oxygen free radicals generated during the runaway inflammatory reaction. Finally, zinc is involved in the metabolism of proline by inhibiting its intracellular transport and the down-regulation of CD4 caused by this amino acid. Zinc inhibits the production of nitric oxide.

Another mineral, gallium, is also present in high concentrations. Gallium is known to down-regulate the production of interleukin-6, TNF alpha and other cytokines present in the inflammatory reaction.

Finally, the high selenium concentration of the plant could lower the level of interleukin-8, regulate the concentration of interleukin-2 and stimulate the production of CD4 lymphocytes.

The plant is extremely rich in vitamins A and E, one of which, when supplemented, is known to reduce morbidity and mortality in viral infections, including HIV, while the other is a powerful antioxidant.

Note also that *Artemisia annua* herbal tea is low in sodium, rich in potassium and contains all essential amino acids including arginine.

All of these active biomolecules form a polytherapy and taking *Artemisia annua* in totum could improve on the one hand exhausted adaptive immunity and on the other hand, modulate the runaway inflammatory response during COVID-19 infection, as this plant has already proven in other serious viral and parasitic infections.