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

Concept note

Dr Catherine Poisson-Benatouil

Anaesthesiologist - Intensive Care Physician
Maison de l'Artemisia Pointe-Noire | Republic of Congo
contact@maison-artemisia.org

assisted by

Jean-Luc Galabert
Consultant
Inter-Culturel design and project management office - Nyamata | Rwanda
etudes@inter-culturel.net

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, human cytomegalovirus) and antiHIV in vitro thanks to the flavonoids, quercetin and dicaffeoylquinic acids it contains. These molecules have been shown to inhibit the enzymaticactivity of CLPro (Chymotrypsin-like protease), an enzyme 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 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.

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

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 not the main immune response (phagocytosis by macrophages and polymorphonuclear neutrophils). There is little to no increase in the polymorphonuclear neutrophil count in clinical studies except in the late stage at the end of the ICU stay.

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, can directly destroy foreign particles, .

The other population of activated lymphocytes is made up of Tfh cells (T follicular helper cells), specific to their 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 382:727-733

Chen N, Zhou M, Dong X et al
Epidemiological features of patients infected with 2019 novel coronavirus in Wuhan, China
Lancet 2020 395:497-506

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 *A. 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 *A. 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 gamma interferon.

A. 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 *A. 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.

Thomas Efferth
Beyond malaria: The inhibition of viruses by artemisinin-type compounds.
Biotechnology Advances 2018, 36: 1730-1737

Matthew R, Desrosiers, 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 compared the antiviral activities of 7 species of *Artemisia* against a number of viruses including HSV1, poliovirus 1 and respiratory syncytial virus. Results showed that the antiviral activity of *A. annua* was strong and, in some cases, superior to that of Acyclovir. Quercetin seemed to be responsible for reduction in viral activity and concentration.

Mehrangiz Khajeh Karamoddini, 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

Thomas Efferth, Marta di Romero, Dana G. Wolff, Thomas Stamminger, Jose J.G.Marin, and Manfred Marshall
The antiviral activities of artemisinin and artesunate
CID 2008 : 47, (15 September)

Another in vitro study demonstrated the powerful anti-HIV activity of A. *annua* herbal tea. This activity was seemingly not due to artemisinin, but rather it was due tothe dicafeylquinic acids (dicaffeoylquinic acids) in the plant. 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

A. annua contains chalcones and quercetin-3-β-d-glucoside. These molecules are antioxidants and have recognized 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 *A. tschnieviana*, but which is also present in *A. annua*, has a powerful antihemolytic action.

Naqinezhad A, Nabavi SM, Nabavi SF, Erahimzadeh MA.
Antioxidant and antihemolytic activities of flavonoid rich fractions of Artemisia tschernieviana Besser
European Review for Medical and Pharmacological Sciences,
2021 Jul; 16 Suppl 3: 88-94.

Quercetins are bioflavonoids that have antiviral activities against HSV1, HSV2, cytomegalovirus and some types of adenovirus. In the study by Zandi et al. (2011), quercetins expressed 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

Other flavonoids, e.g. casticin, and chrysosplenol 6-D, reduced 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 chrysosplenol D from *Artemisia annua* L. inhibit inflammation in vitro and in vivo
Toxicology and Applied Pharmacology, 17 Apr 2015, 286(3): 151-158

Dicafeylquinic acid / chlorogenic acid

Artemisia sp. and *Artemisia annua* are rich in chlorogenic and other caffeoylquinic acids that 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 *A. annua* have viral inhibition properties superior to other molecules of the plant such as artemisinin or arteannuin B. *Artemisia annua* 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 significantly reduce its infectivity.

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 *A. annua* have the greatest antiviral inhibitory activity against tobamovirus. Sterols such as sitosterol and stigmasterol are responsible for this inhibitory activity in *A. 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 and Beta-sitosterol glucosides

The antiviral β-sitosterols and the β-sitosterol glucosides are also present in *A. annua*. They stimulate the cellular immune response that controls viral replication. This antiviral activity was shown in HIV-infected patients. These sterols stabilized the CD4 level of infected individuals and reduced 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.

A. annua also 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 *A. 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 *A. 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, or poorly regulated.

A. annua herbal tea is thus used effectively 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, suggesting 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, there was an increase in the splenic index (enlargement of the spleen) likely caused by the genesis of CD4 lymphocytes and additional CD8 lymphocytes in the spleen and circulation. DHA (dihydroartemisinin) could increase the proportion of T helper cells and CD8+ T cells, as well as decrease the number of splenic and circulatory 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 than in the untreated model group. Dihydroartemisinin could inhibit the production of anti-ds-DNA antibody and secretion of TNF alpha and improve the pathologic lesion of lupus nephritis in BXSB mice.

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.

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

A. 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 *A. 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 H202 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.

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. *A. 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 *A. 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.

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

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

A. annua is rich in polyphenols that 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 *A. annua*, used as adjuvants for the hepatitis C vaccine, increase the secretion of gamma interferon and interleukin-4. Gamma interferon is a Th1 (Lymphocyte THelper1) cytokine and interleukin-4 is a Th2 (Lymphocyte T Helper 2) cytokine. The secretion of gamma interferon is higher

than that of interleukin-4, suggesting that in this case *A. annua* is more effective in inducing a cellular immune response.

However, the secretion of gamma interferon could also directly facilitate B cell differentiation and, thus, stimulate 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)

The microbiota of the infected 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 significant quantities in *A. annua* is inulin. In addition to its role in the direct stimulation of B lymphocytes, inulin 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, GaryR, Le cleir, Joshua E, Denny, Sarah S et al.
Composition of the gut microbiota modulates the severity of Malaria
Proc Natl Acad Sci 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. *A. 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 *A. annua* herbal tea had shorter bleeding times than those treated with Coartem (artemether/lumefantrine). The latter had lower platelet levels than those in the group treated with *A. 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, also present 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 in *A. annua* 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 occurs with a reduction in the CD4 count. In mice supplemented with selenium for 8 weeks CD4 counts increased in proportion to the dose of selenium 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 a number of reports.

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

Also present in the plant, gallium participates in the regulation of cytokines by down regulating inflammation cytokines including interleukin-6 and TNF alpha.

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

A. 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 phagocytize 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 improved the cellular immune response by increasing CD4 and CD4/CD8 ratio as found in a randomized study in 76 children in India.

Such supplementation also 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

A. 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 also present in measureable 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, virus Influenzae).

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

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

Cortese- Krott MM, KulatokovL, 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 and saponins

The concentration of potassium is very high in *A. annua,* which could facilitate the plant's antioxidant properties. *A. 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, Giancgiacomo 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.

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.

A. annua has known antiparasitic activity but also antiviral activity (anti HSV-1, poliovirus, RSV, hepatitis C anti-virus, dengue virus type 2, hantavirus, human Cyto Mégalo Virus) and antiHIV 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, 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 *A. 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 *A. annua* increase the secretion of gamma interferon 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 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 and *A. 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, which become

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, present in the plant, is known to down-regulate the production of interleukin-6, TNF alpha and other cytokines present in the inflammatory reaction.

Finally, the selenium 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 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 *A. annua* herbal tea is low in sodium, rich in potassium and contains all the essential amino acids including arginine.

All of these active biomolecules form a polytherapy and taking *A. 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.