# Artemisia annua + Zinc for the Treatment of COVID-19

# A Potential Successful Combination Therapy with Ivermectin

**Dr. José Luis Abreu**

**Abstract.** This study uncovers and explains for the first time the active chemical and molecular synergistical activities among Artemisia annua, zinc, hydrogen peroxide and phenols. These collective molecular interactions lead to strong anti-COVID-19 properties of Artemisia annua. This research explores the following topics: Zinc in Antiviral Immunity; Anti-Viral Vaccine Activity of Zinc (Ⅱ); Zinc, Hydrogen Peroxide and Neutrophil Defense; Zinc Oxide; Zinc Targeting Iron; Zinc and COVID-19; Phenols, Zinc and The Fenton Reaction; Artemisia annua Phenols; Hydrogen Peroxide; Effects of Hydrogen Peroxide on Plasma Membrane and Calcium Mobilization; Hydrogen Peroxide in Vascular Endothelial Cells; Artemisia annua and Hydrogen Peroxide; Zinc Ionophores & Metal Chelation by Artemia annua; Combination Therapies; Ivermectin and Viral Inhibition.

**Keywords.** Artemisia annua, Zinc, Hydrogen Peroxide, Phenols, COVID-19.

---

## Introduction: Zinc in Antiviral Immunity

It is now understood, according to Read et al (2019), that zinc is the second-most abundant trace metal in the human body after iron, and an essential component of protein structure and function. Importantly, zinc is a structural constituent of ~750 zinc-finger transcription factors enabling gene transcription, and is a catalytic component of approximately 2000 enzymes, encompassing all 6 classes (hydrolase, transferase, oxido-reductase, ligase, lyase, and isomerase). Zinc is biologically essential for cellular processes, including growth and development, as well as DNA synthesis and RNA transcription. It has been clearly established that zinc deficiency results in a compromised immune system.

Read et al (2019) studied in detail the role of zinc as an essential micronutrient that is required to mount an effective antiviral response. Zinc possesses direct antiviral properties. It is also critical in generating both innate and acquired (humoral) antiviral responses. Zinc is an integral component of many viral

enzymes, proteases, and polymerases, highlighting the importance of regulating cellular and systemic zinc distribution to inhibit viral replication and dissemination.

Zinc homeostasis and viral infection was analyzed by Read et al (2019). They found out that systemic and intracellular zinc are tightly regulated, such that free zinc ions (Zn2+) represent a minimal fraction of total cellular zinc (~0.0001%). Most of the zinc remains bound to zinc-binding proteins such as serum albumin or intracellular metallothionein proteins, where it can be transferred to zinc-binding enzymes and transcription factors, as necessary. Zinc transport is principally mediated by 2 groups of proteins: the ZnT [solute-linked carrier 30 (SLC30A)] family, which is responsible for efflux of zinc outside the cell or influx into organelles, and the ZIP [Zrt- and Irt-like proteins (SLC39A)] family of proteins, which performs the opposite role, transporting zinc into the cytoplasm from extracellular sources or cellular organelles.

Another line of research for Read et al (2019) was the relation of metallothioneins, zinc homeostasis, and antiviral activity. They identified metallothioneins as small, cysteine-rich proteins capable of binding divalent cations such as zinc and copper. As vessels for much of the labile intracellular zinc pool, metallothioneins possess numerous functions through their ability to bind and release metals from their thiol groups. These include storage and transfer of zinc ions and heavy metal detoxification, as well as involvement in oxidative stress, apoptosis, and immune responses. Metallothionein expression is extremely responsive to zinc, and therefore serves as an ideal indicator of an individual's zinc status. Upon taking a zinc supplement, for example, an increase in protein-bound zinc in the bloodstream is internalized by cells in various tissues and organs through the ZIP transporters. In response to increased intracellular zinc, the metal-responsive transcription factor (MTF1) becomes active and binds the metal responsive element in metallothionein gene promoters to upregulate their transcription (Read et al, 2019).

Oxidative stress, as explained by Read et al (2019), induces zinc release from metallothioneins as a mechanism to reduce reactive oxygen species generated by mitochondrial dysfunction or viral infection. Zinc released from metallothioneins binds MTF1 to stimulate additional metallothionein expression. It should be noted that metallothioneins, although highly responsive to zinc, have also long been classified as interferon stimulated genes. are immunostimulatory cytokines secreted from infected cells and nearby immune cells that induce the expression of hundreds of antiviral genes. They possess

diverse roles including chemoattraction, immune cell activation, and direct antiviral activity. Interferon stimulates an influx of zinc into the target cell, as is the case with some inflammatory cytokines such as IL-6, which in turn drives metallothionein expression.

It was observed by Read et al (2019) that research data has suggested that metallothioneins are either 1) directly antiviral, potentially by sequestering zinc away from viral metalloproteins such as HCV NS5A (Tellinghuisen et al, 2004), or 2) indirectly antiviral by acting as zinc chaperones and facilitating antiviral signaling. Further, metallothioneins possess antiviral properties against other viruses as well, as demonstrated in an antiviral screen of 380 human ISGs performed by Schoggins et al. (Schoggins et al, 2011). Read et al (2019) noted overexpression of multiple members of the metallothioneins family inhibited replication of flaviviruses including yellow fever virus and HCV, as well as the alphavirus Venezuelan equine encephalitis virus. In addition, they reported that severe acute respiratory syndrome (SARS) coronavirus RdRp template binding and elongation was inhibited by zinc inVero-E6 cells. The antiviral properties of zinc are certainly virus-specific, but it would appear that zinc ion availability plays a significant role in the antiviral efficacy of zinc (Eby, 1997 in Read et al, 2019).

Read et al (2019) concluded that the role of zinc as an antiviral can be separated into 2 categories:

- Zinc supplementation implemented to improve the antiviral response and systemic immunity in patients with zinc deficiency, and
- Zinc treatment performed to specifically inhibit viral replication or infection-related symptoms zinc ionophore.

During a virus infection, reprogramming of the host cell occurs for mainly two reasons (Zhao et al 2003, in Ishida, 2018). First, the virus needs to establish optimal conditions for replication to ensure efficient production of progeny virus, secondly, the virus must interfere with the host cell antiviral defense mechanisms to maximize the likelihood of escape and spread of the progeny virus. During virus infection, there are virus-specific processes within the virus replicative cycle or virus-infected cell that attract targets for chemotherapeutic intervention such as virus adsorption and entry into the host-cell. Thus, one attractive approach to the prevention of infection is inhibition of virus replication (Ishida, 2018).

Entry of the virus into the host cell is mediated by the viral envelope glycoprotein (GPC). It is likely that for zinc-binding domain in the virus envelope glycoprotein, the zinc-mediated anchoring of stable signal peptide (SSP) contributes to positioning the ectodomain loop of SSP relative to the G2 ectodomain to modulate membrane fusion. Small molecule compounds that target the pH-sensitive SSP-G2 interface in the ectodomain of GPC have been shown to inhibit pH-induced activation of membrane fusion and prevent virus entry (Briknarova, 2011). The coronavirus (CoV) S protein requires cleavage by host cell proteases to mediate virus-cell and cell-cell fusion which many strains of the murine coronavirus mouse hepatitis virus (MHV) have distinct, S-dependent organ and tissue tropisms despite using a common receptor, suggesting that they employ different cellular proteases for fusion (Phillips, 2017 in Ishida, 2018). Thus, the use of cellular zinc metalloproteases is effective to stop virus entry and coronavirus fusion. Zn2+ is strong inhibitor of the fusion step of several viruses. It was found that the degree of inhibition depends upon the zinc concentration (Ishida, 2018).

Ishida (2018) concluded that viruses depend on a host cell for their protein synthesis, that the virus must first bind to the host cell, and then the virus enters in the cytoplasm in which the genome is liberated from the protective capsid, either in the nucleus or in the cytoplasm. The use of cellular zinc metalloproteases is effective to control virus entry and coronavirus fusion and increasing the intracellular Zn2+ concentration with zinc-ionophores can efficiently impair the replication of a variety of RNA viruses, including SARS-CoV.

In has been reported that Artemisia annua is one of the three plants richest in zinc. It is considered a super accumulator of zinc (Poisson-Benatouil, 2020).

**Anti-Viral Vaccine Activity of Zinc (Ⅱ)**

Ishida (2019) investigated the anti-viral vaccine activity of Zn2+ ions for viral prevention, pathogenesis processes, and ROS generation causing oxidative stress. Viruses are small protein capsid that harbor genetic information. In the case of enveloped viruses, an additional lipid bilayer surrounds the capsid that enveloped viruses can spread via two distinct routes, either through the cell-free aqueous environment or, alternatively, by remaining cell associated and being passed on by direct cell-cell contact. Zinc has a rather low toxicity and influences apoptosis by acting on several molecular regulators of programmed cell death, including caspases and proteins from the Bcl and Bax families.

Zinc induced anti-virus activity may be enhanced for T cell division, maturation and differentiation, lymphocyte response to mitogens, programmed cell death of lymphoid and myeloid origins, gene transcription, and biomembrane function. The induced zinc-finger antiviral protein (ZAP) during viral infection is little known, however, mutational analysis of the human ZAP promoter revealed that multiple interferon stimulated response elements (ISREs) distal to the transcription start site serve redundantly to control interferon regulatory factor 3 (IRF3)-dependent induction of ZAP transcription. The ability to create a zinc finger nuclease (ZFN) vaccine that can prevent and eliminate persistent viral infections is a long way from being reached. Viral pathogenesis is the process by which an infection leads to a disease with pathogenic mechanisms of viral disease including viral entry, local replication, and spread to organs. Accordingly, the zinc induced Zn2+ ion coordinated activity results in regulation of viral growth and may lead to virus death in host cell-virus interaction during the process of pathogenesis (Ishida, 2019).

ZAP inhibits the spreading of viral infection. By using zinc oxide nanoparticles the treatment of viral infections with viral spreading can be achieved with improved therapeutic effects. ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) within viruses of viral entry, viral replication, and viral spread are generated in all situations. ROS induce several cellular effects, including cell cycle progression, apoptosis, DNA damage, senescence, and neurodegeneration. ROS in virus pathogenesis play an important role in cell signaling and regulate hormone action, growth factors, cytokines, transcription, apoptosis, immunomodulation, and neuromodulation, leading to chronic oxidative stress. Oxidative stress occurs in most of the viral infections. The body antioxidant system becomes weaker as the virus progresses and the imbalances inside and outside the cell induce the cell to undergo a programmed cell death. The antioxidant components lead to an excess storage of H2O2, which further increases the hydroxyl radicals and lipid peroxide that signal the cell to undergo a programmed cell death (Ishida, 2019).

The zinc-associated vaccine activity mechanism against viruses has shown that the anti-viral vaccine activity of released Zn2+ ions from zinc solutions and ZnO NPs, may be enhanced by Zn2+ ion-induced Zn2+ ions-coordinated adapted immunity, viral growth regulation, and viral apoptosis and death (Ishida, 2019).

**Zinc, Hydrogen Peroxide and Neutrophil Defense**

$H2O2 + Zn \rightarrow ZnO + H2O$

This is an oxidation-reduction (redox) reaction:

$Zn^{0} - 2\ e^{-} \rightarrow Zn^{II}$ (oxidation)

$2\ O^{-I} + 2\ e^{-} \rightarrow 2\ O^{-II}$ (reduction)

*Zn* is a reducing agent, $H_2O_2$ is an oxidizing agent.

Reactants: Hydrogen peroxide (H2O2) and Zinc (Zn)

Products: Zinc oxide (ZnO) and Water ($H_2O$)

Hasan, Rink and Haase (2012) from RWTH Aachen University Hospital, Medical Faculty, Institute of Immunology in Germany, observed that neutrophil granulocytes, also known as polymorphonuclear leukocytes (PMN), are the largest population among the cells of the innate immune system and an essential component of the first line of defense against invading pathogens. Once PMN arrive at the site of infection, they employ different strategies to kill pathogens. Primarily, PMN engulf microorganisms and ingest them by phagocytosis. Upon internalization of pathogens into phagocytic vesicles, the latter are fused with lysosomes containing proteins with anti-pathogenic activity. These proteins include NADPH oxidase, an enzyme capable of producing high amounts of reactive oxygen species (ROS).

PMN cast out their DNA, chromatin and granule proteins into the extracellular space, forming a matrix known as neutrophil extracellular traps (NET). These NETs capture and kill pathogens, contributing to clearance of the infection. This specialized form of cell death, so-called NETosis, is NAPDH oxidase-dependent, and differs both biochemically and morphologically from necrosis and apoptosis. It was also established that H2O2 can promote NET production (Hasan, Rink and Haase, 2012).

Mimicking the synthesis of ROS by NADPH oxidase through addition of exogenous H2O2 leads to elevated free Zn2+. This further supports an oxidative mechanism for generation of the zinc signal. In addition, treatment with H2O2 resulted in the formation of NETs, indicating that generation of ROS is

sufficient for triggering all signaling pathways necessary for NET formation. Moreover, chelation of the zinc signal by TPEN (tetrakis 2-pyridylmethyl-ethylenediamine) inhibited the H2O2-induced NET release, confirming that Zn2+ is essential for NET formation in response to H2O2, as well as PMA (activator 12-myristate 13-acetate) (Hasan, Rink and Haase, 2012).

The conclusions of Hasan, Rink and Haase (2012) showed that zinc signals are an essential component of the ROS-dependent signal transduction leading to NETosis. Also, it was found out that H2O2 can promote NET production.

At the University of Sydney, Tang et al (2016) studied the neutrophil activation in respiratory viral infections. Their study aimed to investigate if neutrophils can be directly activated by respiratory viruses. Neutrophils appear to be implicated during viral infections; however, the immune responses that regulate virus-induced exacerbations and, in particular, neutrophilic inflammation remain poorly defined.

Tang et al (2016) reported that neutrophils contain a range of receptors that recognize pathogen-associated molecular patterns (PAMPs) present on viruses. The majority of respiratory viruses are RNA viruses that can be detected by toll-like receptors (TLRs). Studies have already described the expression neutrophils. It was found that neutrophils contain the relevant components to detect and respond to both bacteria and viruses.

It was proposed by Tang et al (2016) that airway epithelium or smooth muscle, both of which are known to be significantly modulated in the context of respiratory viral infections, may play an important role in initiating a cascade of events during the infection that results in extensive neutrophilic inflammation. Alternatively, it is not activation of neutrophils that is significant during exacerbations, rather the resolution or regulation of neutrophilic inflammation following infection that may be the determining factor in exacerbations. Animal models of lung viral infection clearly show that inhibition of lung neutrophilia causes detrimental outcomes, and defective resolution of inflammation is hypothesized to contribute to the chronicity of the disease.

**Zinc Oxide**

Zinc oxide nanoparticles (ZnO-NPs) have been demonstrated to exert antimicrobial activities against various human pathogens. Most studies have focused on their inhibitory actions on bacterial infections, and there is limited studies evaluating the interaction between ZnO-NPs and viruses. In a recent work by Ghaffari et al (2019), they found a strong inhibitory effect of ZnO-NPs and polyethylene glycol (PEG)-coated ZnO-NPs (ZnO-PEG-NPs) on HSV-1. In this line, they conducted a study to investigate the effects of ZnO-NPs and ZnOPEG-NPs on the replication of H1N1 influenza virus, which are amongst the most challenging viruses that threaten human health. Taken together, their study indicated that PEGylated ZnO-NPs could be a novel, effective, and promising antiviral agent against H1N1 influenza virus infection, and future studies can be designed to explore the exact antiviral mechanism of these nanoparticles (Ghaffari et al, 2019).

ZnO NPs have become one of the most popular metal oxide nanoparticles in biological applications due to their excellent biocompatibility, economic, and low toxicity. ZnO NPs have emerged a promising potential in biomedicine, especially in the fields of anticancer and antibacterial fields, which are involved with their potent ability to trigger excess reactive oxygen species (ROS) production, release zinc ions, and induce cell apoptosis. In addition, zinc is well known to keep the structural integrity of insulin. So, ZnO NPs also have been effectively developed for antidiabetic treatment (Jiang, Pi, and Cai, 2018).

Kumar et al (2018) studied the virostatic potential of zinc oxide (ZnO) nanoparticles on capsid protein of cytoplasmic side of chikungunya virus. Their results clearly indicated that zinc oxide nanoparticles interacting with viruses exhibit antiviral activity and this could be new possible antiviral drug against that type of pathogens.

Yang et al (2017) have reported that zinc oxide nanoparticles (ZnO NPs) have many biomedical applications such as chemotherapy agents, vaccine adjuvants, and biosensors. They found aout that surface functionalization-specific binding of coagulation factors by zinc oxide nanoparticles delays coagulation time and reduces thrombin generation potential in vitro.

**Zinc Targeting Iron**

Donangelo et al (2002) found evidence showing that zinc and iron interact competitively during intestinal absorption. There is evidence that excess zinc inhibits iron uptake. Because zinc and iron interact during absorption and, possibly also during metabolism, supplementation of only one of the two may affect the status of the other nutrient. Supplementation with iron or zinc alone at bedtime for only 6 wk improved iron or zinc status. Iron supplementation did not affect measures of zinc status, but zinc supplementation appeared to further reduce iron status.

Zinc supplementation also alters indices of iron status in a manner consistent with a reduction in iron stores. Plasma transferrin saturation decreases, indicating an inadequate tissue supply of iron. Reduced availability of cell iron for heme synthesis during erythropoiesis. The data suggests that supplemental zinc further impairs the iron status (Donangelo et al, 2002).

Studies performed in humans have shown an inhibitory effect of zinc on iron absorption when both minerals are administrated together in fasting conditions. It had been previously found that zinc administration combined with iron in an aqueous solution leads to the inhibition of iron absorption, which occurs in a dose-dependent way (Olivares et al, 2007).

It has been postulated that there is a common pathway of iron and zinc uptake located in the apical membrane of the intestinal cell. The possibility that zinc can compete with iron for transporters in plasma or in their use by different tissues should be considered. It has been shown that transferrin, the main iron plasma transporter, can also bind zinc. Additionally, zinc can block the iron storage capacity of ferritin (Olivares et al, 2007).

The recognized antagonistic actions between zinc and iron prompted Neves de Brito (2014) to study this subject. The basal serum zinc concentration significantly increased after oral zinc supplementation. However, basal serum iron concentrations and area under the iron curves significantly decreased in the experimental group and remained at the same level throughout the study. In conclusion, the decrease in serum iron was likely due to the effects of chronic zinc administration.

## Zinc and COVID-19

te Velthuis et al (2010) reported that Zn inhibits Coronavirus and RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. the intracellular concentration of free Zn2+ is maintained at a relatively low level by metallothioneins, likely due to the fact that Zn2+ can serve as intracellular second messenger and may trigger apoptosis or a decrease in protein synthesis at elevated concentrations. Interestingly, in cell culture studies, high Zn2+ concentrations and the addition of compounds that stimulate cellular import of Zn2+, were found to inhibit the replication of various RNA viruses, including, respiratory virus. There is information that suggests that intracellular Zn2+ levels affect a common step in the replicative cycle of these viruses.

Positive-stranded RNA (+RNA) viruses include many important pathogens. They have evolved a variety of replication strategies but are unified in the fact that an RNA-dependent RNA polymerase (RdRp) functions as the core enzyme of their RNA-synthesizing machinery. The RdRp is commonly embedded in a membrane-associated replication complex that is assembled from viral RNA, and viral and host proteins. Given their crucial function in the viral replicative cycle, RdRps are key targets for antiviral research. Increased intracellular Zn2+ concentrations are known to efficiently impair replication of a number of RNA viruses, by interfering with correct proteolytic processing of viral polyproteins. te Velthuis et al (2010) showed that corona replication can be inhibited by increased Zn2+ levels, and also demonstrated that this effect may be based on direct inhibition of RdRps.

## Phenols, Zinc and the Fenton Reaction

Phenolic phytochemicals (phenolics) occupy a unique position in the area of natural products due to their ubiquitous distribution throughout the plant kingdom and in products (fruits, vegetables, beverages, herbs, cosmetics and nutraceuticals) consumed by the general population on a regular basis. Phenolics are biosynthesized by plants during normal development and in response to stress conditions such as exposure to UV radiation, pest attack, and wounding. Phenolic compounds are known to provide protection against a wide range of diseases. Chemically, phenolics are defined as a class of aromatic organic compounds with at least one hydroxyl group attached directly to a benzene ring. Phenolics can be chemically grouped into three broad categories: polyphenols (tannins and flavonoids), simple phenols (phenolic acids) and a miscellaneous group (Ferreira et al, 2010).

Polyphenols are described as a group of chemical substances found in plants, characterized by the presence of more than one phenol unit or building block per molecule. Polyphenols serve as antioxidants as they tend to prevent or neutralize the damaging effects of free radicals. They also give flowers, fruits, and vegetables their color. Phenolic acids are chemically defined as carboxylic acid derivatives of phenols. Polyphenols can be arranged into two broad classes: tannins and flavonoids. Tannins are astringent, bitter plant polyphenols that either bind or precipitate proteins. Tannins can be further classified chemically into two main groups, hydrolyzable and condensed. Hydrolyzable tannins decompose in water yielding various water-soluble products, such as gallic acid or ellagic acid, protocatechuic acid and sugars. Flavonoid is a general name for phytochemicals based on a 15 carbon (C6-C3-C6) skeleton. Over 4,500 different flavonoids have been isolated and identified from plants. Flavonoids can be further divided into multiple groups such as flavones, flavonols, flavanones, dihydroflavonols, chalcones, aurones, isoflavonoids, biflavonoids, etc (Ferreiraet al, 2010).

Phenolic acids can be broadly grouped into two subgroups: hydroxylcinnamic and hydroxylbenzoic acids derivatives. The miscellaneous group comprises: lignans, lignins, coumarins, stilbenes derivatives like resveratrol, and other phenolic compounds (Ferreira et al, 2010).

The compounds in which a hydroxyl group is bonded to an aromatic ring are called phenols. Phenols constitute probably the largest group of plant secondary metabolites, varying in size from a simple structure with an aromatic ring to complex ones such as lignins (Aldred, 2009). Plant phenols have been studied for hundreds of years and have acted as the major class of compounds that show great activity viruses. Because of the extensive antiviral activities, phenolic compounds have been widely investigated both chemically and biologically. The distribution of hydroxyl groups and ester group accounts for different antiviral activities of phenolic compounds, and research of these compounds has revealed that phenols have great potential for the development as therapeutic agents against various viruses (Li, 2017).

Currently, it is necessary to discover new and better antivirals with novel mechanisms of action for the treatment of COVID-19. For Roa-Linares et al (2019), seeking for potential effective and selective antiviral treatments, substances obtained from natural products provide unlimited opportunities for new drugs due to their availability and chemical diversity. In this regard, quinones are phenolic-related

secondary metabolites that exhibit diverse pharmacological properties, including antiviral, antimicrobial and anti-inflammatory activities.

Roa-Linares (et al 2019) explained in their research report that the mechanisms of action of quinones are mainly related to their ability to inhibit electron transport and to uncouple oxidative phosphorylation. They can also act as intercalating agents into the DNA double helix, as bioreductive alkylators of biomolecules, and as inducers of reactive oxygen species (ROS). In particular, the activity of quinones has been related to ROS generation.

The oxidative degradation of organic matter by H2O2 in the role of Fe2 + under acidic conditions was first discovered by H.J. Fenton in 1894. Early research on the Fenton reaction was mainly focused on organic synthesis, enzymatic reactions and the cell damage mechanism. It was noted that in the presence of a catalyst, H2O2 can be efficiently decomposed to generate oxidative active substances with strong oxidizing power, and degrade a variety of organic compounds (Liu et al, 2018).

For Liu et al (2018), the first and most popular theory, known as the Haber-Weiss mechanism involves the formation of • OH radical in the process of H2O2 reduction. The hydroxyl radical mechanism was first proposed by Haber and Willstatter in 1932, which revealed the existence and role of free radicals in the reaction system and regarded the essence of the reaction as • OH generated by a catalytic during the chain reaction between Fe2+ and H2O2. Throughout the reaction process, the chain initiation phase consists of a series of single electron transfer reactions between Fe2+ and H2O2, • OH and H2O2, • OOH and H2O2 (reaction 1-4), and the generated oxygen free radicals induce the chain growth process (• OH, • OOH). Based on this theory, many scientists conducted extensive research. George (1947 in Liu et al, 2018) detected the presence of O2-• in the study of the KO2--H2O2 system, noted that the dissolved oxygen in the system significantly inhibits the decomposition of H2O2, and proposed that during the study of the mechanism, the role of dissolved oxygen in the Fenton system cannot be ignored. Thereafter, Barb and Weiss (1949 in Liu et al, 2018) introduced O2-• into the reaction system, and amended the reaction mechanism, that is, the dissolved oxygen in the system is related to the generation and reduction process of Fe3+, and proposed the reaction (5). Regarding the oxygen generation in reaction (5), Barb & Baxendale (1951 in Liu et al, 2018) studied the generation mechanism and kinetic characteristics of oxygen in the Fenton system and finalized the radical

mechanism for the Fenton reaction, with the conclusion that the reaction of the system is composed of the following elementary reactions:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO\cdot \quad (1)$$

$$HO\cdot + H_2O_2 \longrightarrow H_2O + HOO\cdot \quad (2)$$

$$H_2O_2 + HOO\cdot \longrightarrow H_2O + O_2 + HO\cdot \quad (3)$$

$$Fe^{2+} + HO\cdot \longrightarrow Fe^{3+} + OH^- \quad (4)$$

$$Fe^{3+} + O_2^{-}\cdot \longrightarrow Fe^{2+} + O_2 \quad (5)$$

$$HO\cdot + H_2O_2 \longrightarrow H_2O + H^+ + O_2^{-}\cdot \quad (6)$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + HOO\cdot + H^+ \quad (7)$$

$$H_2O_2 + H^+ + O_2^{-}\cdot \longrightarrow H_2O + O_2 + HO\cdot \quad (8)$$

$$Fe^{2+} + HO\cdot + H^+ \longrightarrow Fe^{3+} + H_2O \quad (9)$$

$$HO\cdot + HO\cdot \longrightarrow H_2O_2 \quad (10)$$

The kinetics of the Fenton reaction is complex and extensively described in the literature. Essentially, a transition-metal (Fe2+) in acidic aqueous medium catalyzes the decomposition of an oxidant (H2O2) into unselective free radicals (HO·) as in the reaction Fe2++H2O2→Fe3++OH-+HO·. In turn, these radicals oxidize organic elements by generating organic radicals. The multistep kinetics of the Fe2+ / H2O2 chemistry is affected by numerous factors. In the Fenton scheme, phenol is hydroxylated to hydroquinone and then oxidized to quinone before its further oxidation to aliphatic acids (López et al, 2017).

Friedrich et al (2012) studied the mechanistic implications of zinc(ii) ions on the degradation of phenol by the Fenton reaction. Fenton showed that a mixture of H2O2 and Fe2+ had very strong oxidizing properties. The Fenton reaction is an important source of hydroxyl radicals, which oxidize organic substances. A means of accelerating the rate of the Fenton reaction is via the use of phenolic compounds, especially dihydroxybenzene (DHBs), which have the ability to reduce Fe3+ to Fe2+. Hamilton et al. (1966) were the first to report that catalytic amounts of dihydroxybenzene (catechol or 1,4-hydroquinone), the primary initial intermediates in phenol degradation were able to increase the degradation rate of aromatic compounds in Fenton reactions. The same effect is observed when catechol (phenolic group) is added to the reaction medium. Fe2+ forms a 1:1 complex with catechol

that decomposes the ortho-semiquinone radical and Fe3+ ion. The semiquinone radical is unstable and is oxidized by another Fe3+ ion, leading to 1,2-benzoquinone. The 1,2-benzoquinone formed can interact with the superoxide ion to reform the semiquinone radical and molecular oxygen. This redox cycle represents the Hamilton catalytic cycle (Figure 1).

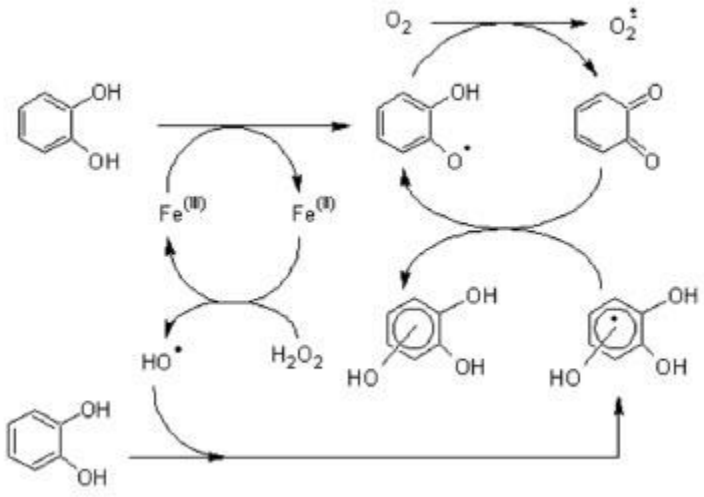



**Figure 1. The Hamilton catalytic cycle. Reference: Friedrich et al (2012)**

One of the first intermediates formed in the reaction, catechol, can reduce Fe3+ to Fe2+ and, in the presence of H2O2 initiates an efficient catalytic redox cycle. In the initial stages of the reaction, this catechol-mediated cycle becomes the principal route of thermal degradation of phenol and its oxidation products. The Zn2+ ion addition enhances the persistence time of catechol, probably by stabilization of the corresponding semiquinone radical via complexation (Friedrich et al, 2012)

The research carried out by Friedrich et al (2012) confirmed the existence of an effect of Zn2+ ion on persistence time of the formed intermediates on the phenol degradation by the Fenton reaction. Catechol, one of the main initial intermediates in the Fenton oxidation of phenol, can complex with Fe3+. This complex is capable of reducing Fe3+ to Fe2+, by catalyzing the overall process of oxidation. In the initial stages of the Fenton reaction, the presence of zinc ions exerts a beneficial effect on the phenol degradation since it enhances the persistence of catechol, probably via stabilization of the corresponding semiquinone radical. These results provided further indications that the reduction of Fe3+ to Fe2+ in the presence of H2O2 and catechol is an important catalytic pathway for the Fenton

reaction in our system. The results also suggested that zinc ions do not interfere with the formation of HO· radicals.

Chen et al. (1997 in Liu et al, 2018) studied the phenol degradation process and presented the corresponding degradation path, with improvement on the degradation mechanism of aromatic compounds and the practicability of the Fenton system. The author believed that the phenol is first attacked by • OH to generate the radical of dihydroxy cyclohexadiene (DHCD•), after that DHCD• will be dehydrogenized into hydroquinone (HQ). HQ with strong reduction ability can react quickly with Fe3+ to produce semiquinone radical (SQ •), and SQ • will continue to reduce Fe3+ into benzoquinone (BQ), at last BQ will be reduced into SQ • by DHCD •. In the above process, the quinone is an intermediate which will accelerate the circulation of Fe3+/Fe2+ and promote the generation of Fe2+. The whole process is shown as an autocatalytic process (Figure 2). The Duesterberg research group [46] investigated the autocatalytic process of the oxidation on the hydroxy acid by the Fenton system. Based on the Pignatello research model, they revised and added some reactions, adjusted the rate constants of radical reactions, and established a kinetic model of the oxidation on the hydroxy acid by the Fenton system. The results also proved the role of quinone intermediates in the catalytic degradation process of aromatic compounds (Figure 2).

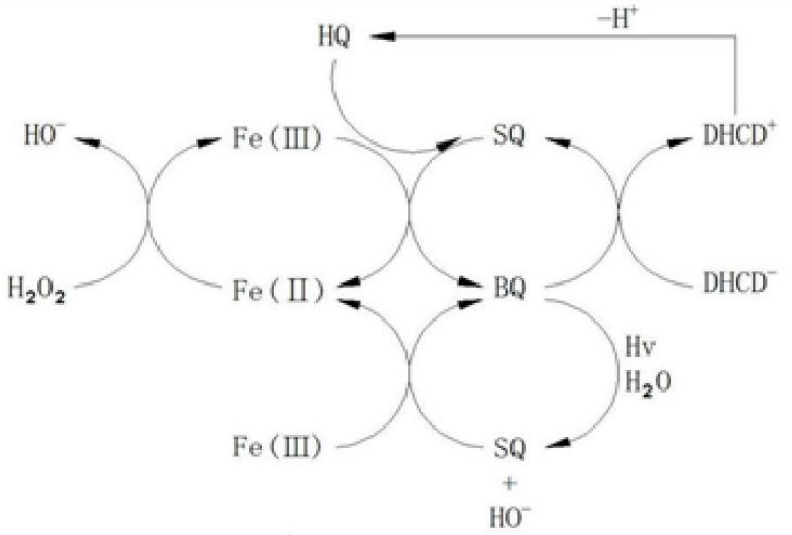



**Figure 2. Quinone intermediates electron transfer in the process of phenol oxidation (Liu et al, 2018)**

Ahmed (2019) reported in his doctorate thesis that H2O2 treatment resulted in significant increases in [Zn2+], providing evidence that ROS acts to elevate [Zn2+]. Sensitivity to ROS implicates redox-sensitive Zn2+-storage proteins, such as metallothioneins, as a mechanism by which Zn2+ is increased. Metallothioneins are cysteine-rich proteins which transiently bind heavy metals such as Zn2+. Metallothionein has been found to have a higher affinity for Zn2+ than most other Zn2+ binding proteins. Zn2+ binds to cysteines, producing a sulphur and Zn2+ network. The proposed mechanism of Zn2+ binding and release from and to the metallothionines is redox dependent. Sulphur and Zn2+ complex can undergo oxidation and reduction to release and bind Zn2+. This mechanism enables Zn2+ to be released, elevating the [Zn2+] concentration. H2O2 provided evidence that elevation of ROS induces elevated [Zn2+], possibly in a redox-dependent manner involving metallothionines. The data demonstrated that increases in ROS is attributable to Zn2+ and not Ca2+.

In an interesting research activity, Wallen, Bacsa and Scarborough (2015) studied the hydrogen peroxide complex of zinc. Zn(II)-(H2O2) complexes have been implicated in kinetic and computational studies but have never been observed. Accordingly, H2O2 has been described as potential ligand. They reported the first Zn(II)- (H2O2) adduct, which is made possible by incorporating intramolecular hydrogen-bonding interactions with bound H2O2. The Zn(II)-H2O2 complex decays in solution by a second-order process that is slow enough to enable characterization of this species. This report speaks to the intermediacy of Zn(II)-(H2O2) adducts in chemistry and biology and opens the door to exploration of these species in correlation with Artemisia annua.

**Artemisia annua Phenols**

Ivanescu et al (2010) identified and studied eighteen polyphenolic compounds in the aerial parts of Artemisia annua. The substances were one hydroxybenzoic acid, six cinnamic acid derivatives, four quercetin glycosides, and seven aglycones of flavonol and flavone type.

Weathers et al (2014) reported the following phenolic acids: Rosmarinic ((2"R")-2-[[(2"E")-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl) propanoic acid) and chlorogenic ((1S,3R,4R,5R)-3-{[(2Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid) acids are strong antioxidants found in a wide variety of A. annua cultivars. In Caco-2 studies, these acids significantly inhibited activity of CYP3A4, one of the hepatic P450s responsible for metabolism of artemisinin to deoxyartemisinin, an inactive form of the drug.

These and other phenolic acids are present in A. annua tea infusion. Both phenolic acids have an IC50 of about 65 μmol/L and also significantly reduced secretion of cytokines IL-6 and IL-8, and thus enhanced antiviral activity while reducing inflammation.

Ştefanache et al (2016) performed a phenolic compounds assessment. The extraction of phenolic compounds was made using 100% methanol under ultrasound assisted extraction. They identified the following phenolic compounds: rutin, chlorogenic acid, hyperoside, luteolin-7-glucoside and cynarin, caffeic acid, p-coumaric acid, hyperoside, isoquercitrin, and luteolin-7-glucoside. Cynarin was the major phenolic compound in all A. annua samples. They concluded that phenolic acids are the dominant constituents.

32 polyphenols characterized in various parts of plant tissue, including flowers, leaves, stems and roots were detected for the first time using liquid chromatography–tandem mass spectrometry (LC/MS/MS) by Song et al (2016). The roots contained the largest concentration of identified components, while the flowers contained the least. The antioxidant capacity and radical cation-scavenging activities were highest in the roots and lowest in the flowers. Their findings are well correlated and suggest that the antioxidant capacities principally depend upon the polyphenol concentrations in each part of the plant.

The high antioxidant activity of A. annua extract is most likely due to its high phenolic content, according to Ferreira et al (2010). Over 50 different phenolic compounds belonging to five major groups (flavones, flavonols, coumarins, phenolic acids, and a miscellaneous group have been reported from A. annua (Figure 3).

The prominent coumarins identified by Ferreira et al (2010). from A. annua are coumarin, aesculetin (6,7-dihydroxycoumarin), iso-fraxidin (7-hydroxy-6,8-dimethoxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin), scopolin (7-β-D-glucopyranoside-6-methoxycoumarin), and tomentin (5-hydroxy-6,7-dimethoxycoumarin). The main components of A. annua were recently identified by HPLC-MS as quercetinglucoside, flaviolin, rhamnetin, chrysoplenol D, and pilloin, although the HPLC-UV data suggested that when detection was done at 335 nm more than 40 components, including chlorogenic acid, were present.

The structures of the 11 prominent flavones and 29 flavonols reported from A. annua were reported by Ferreira et al (2010). A highly specific feature of A. annua is the presence of significant quantities of

structurally diverse polymethoxylated flavonoids. In addition, other phenolic compounds such as 2,4-dihydroxy-6-methoxy-acetophenone, 5-nonadecyl-3-O-methyletherresorcinol, 2,2,6-trihydroxychromene, and 2,2-dihydroxy-6-methoxychromene have also been isolated from A. annua.

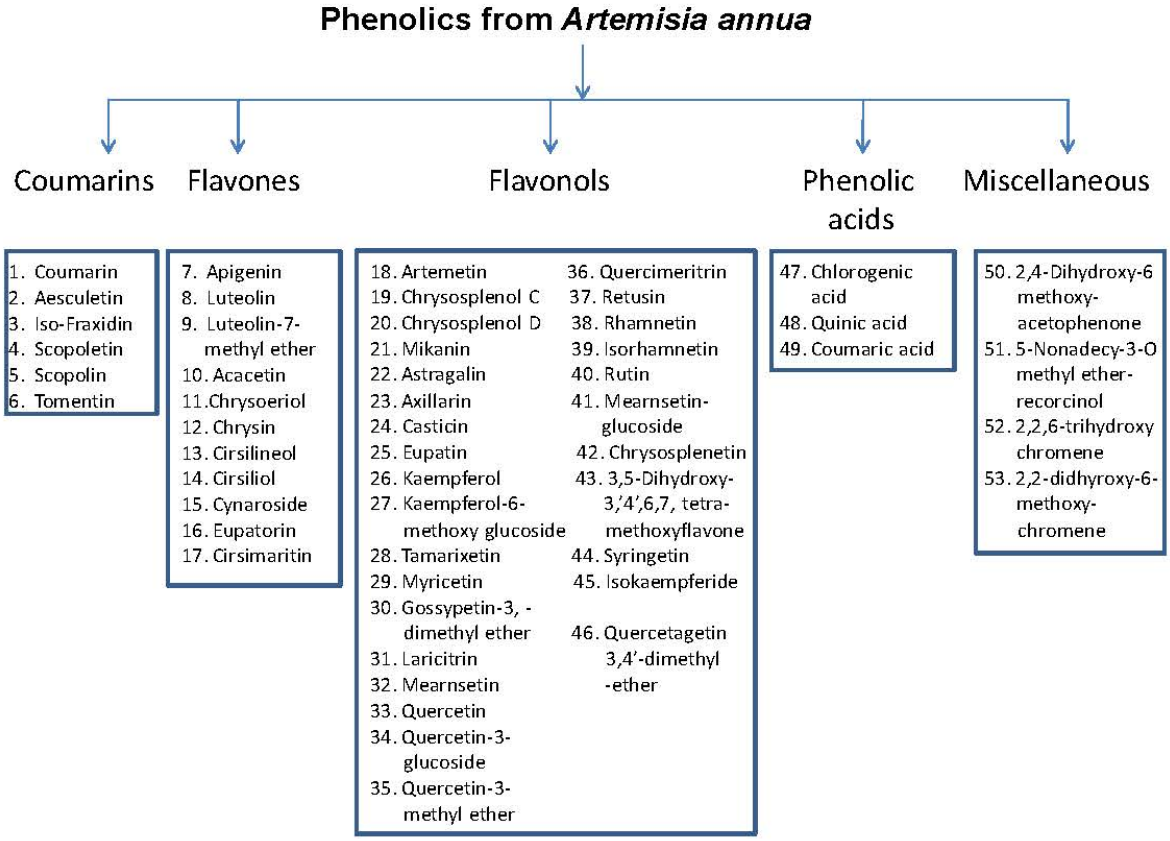



**Figure 3. Major phenolics from Artemisia annua. Ferreira et al (2010).**

Artemisia 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 (Poisson-Benatouil, 2020).

**Hydrogen Peroxide**

In a letter to the editor, Caruso et al (2020) reported that to prevent the spread of the virus, in February 2020, the Italian government issued a recommendation, among the methods of sanitizing the environments, for the use of 0.5% hydrogen peroxide. Hydrogen peroxide is already widely used as an environmental, surgical disinfectant. SARSCoV-2 is spread by human-to-human transmission; the

infection is estimated to have an average incubation period of 6.4 days and a base reproduction number of 2.24–3.58. Furthermore, scientific studies have proven that the virus persists for 2 days on the mucous membranes of macaques before the subsequent spread of the virus to the lower respiratory tract. This delay represents a window of therapeutic opportunity.

The efficient inactivation of coronaviruses on inanimate surfaces using hydrogen peroxide (H2O2 0.5% for 1 minute) has been assessed. Based on these findings, and after reviewing the current literature concerning hydrogen peroxide, Caruso et al (2020) propose that hydrogen peroxide, as an antiseptic agent, could play a pivotal role in reducing the hospitalization rate and COVID-19–related complications. The antiseptic efficacy of hydrogen peroxide 3% against SARSCoV-2 on oral and nasal mucosa can be reasonably hypothesized.

The antiseptic action is due not only to the known oxidizing and mechanical removal properties of hydrogen peroxide but also to the induction of the innate antiviral inflammatory response by overexpression of Toll-like receptor 3 (TLR3). Thus, the overall progression of the infection from the upper to the lower respiratory tract can be reduced (Caruso et al, 2020).

Caruso et al (2020) advise an off-label use of H2O2 3% and 1.5 % (10 volumes) by oral and nasal washing respectively, performed immediately after the onset of the first symptoms and the presumptive diagnosis of COVID-19 and during the illness in home quarantine or by hospitalized patients not requiring intensive care. They propose a regimen of gargling 3 times per day for disinfection of the oral cavity and nasal washes with a nebulizer twice daily (due to a greater sensitivity of the nasal mucosa). Hydrogen peroxide (H2O2) is safe for use on the mucous membranes as gargling or as a nasal spray.

Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress was studied by Sies (2017). Hydrogen peroxide emerged as the major redox metabolite operative in redox sensing, signaling and redox regulation. H2O2 is recognized as being in the forefront of transcription-independent signal molecules. As a messenger molecule, H2O2 diffuses through cells and tissues to initiate immediate cellular effects, such as cell shape changes, initiation of proliferation and recruitment of immune cells. It became clear that H2O2 serves fundamental regulatory functions in metabolism beyond the role as damage signal.

The metabolic and regulatory role of this oxygen metabolite has been increasingly recognized. It serves as a key molecule in the Third Principle of the Redox Code, which is: “Redox sensing through activation/deactivation cycles of H2O2 production linked to the NAD and NADP systems to support spatiotemporal organization of key processes” ·H2O2 occurs in normal metabolism in mammalian cells and is a key metabolite in oxidative stress (Sies, 2017).

It took until 1970 that the physiological occurrence of H2O2 as a normal attribute of mammalian metabolism was demonstrated. A timeline shows the appearance, on the horizon of research, of the Fenton reaction and the naming of peroxidases and catalase in the 19th century. Glutathione peroxidase (Mills), superoxide dismutase (McCord and Fridovich) and peroxiredoxins (Rhee) were further milestones. The transport of H2O2 across membranes by water channels (Henzler and Steudle), by specific aquaporins designated as peroxiporins, concluded that development in the 20th century (Sies, 2017).

Considering the level of the whole organism, mucosal barrier tissues participate in immune defense against infection. The release of nanoto submicromolar H2O2 was shown to disrupt the tyrosine phosphorylation network in several pathogens as a host-initiated antivirulence strategy (Sies, 2017).

H2O2 is a protonated form of O2 (2−) and is produced in biological systems by the dismutation of superoxide anion in a reaction carried out by the enzyme superoxide dismutase (SOD) in the following manner Vilema-Enríquez (2016):

$$M^{(n+1)+}\text{-SOD} + O_2^- \longrightarrow M^{n+}\text{-SOD} + O_2$$

$$M^{n+}\text{-SOD} + O_2^- + 2H^+ \longrightarrow M^{(n+1)+}\text{-SOD} + H_2O_2,$$

$$\text{where M} = \text{Cu}(n = 1); \text{Mn}(n = 2); \text{Fe}(n = 2); \text{Ni}(n = 2).$$

H2O2 is also a soluble lipid and strong oxidizing agent that has been suggested to diffuse throughout the cell membrane via some aquaporins like aquaporin-8, AtTIP1;1, andAtTIP1;2. H2O2 is also a hypochlorous acid precursor. This ROS reacts in the presence of transition metals like cupper or iron and produces the hydroxyl radical, a powerful reactive and toxic compound (Vilema-Enríquez, 2016).

One of the preferred targets for H2O2 is the DNA; it produces single- or double-stranded DNA breaks as well as DNA cross links, in addition to purine, pyrimidine, or deoxyribose modifications. Changes in DNA are usually repaired by the cell, but when persistent DNA damage occurs, then replication errors, genomic instability, activation of oncogenes, and inactivation of tumor suppressor genes might emerge (Vilema-Enríquez, 2016). Increasing evidence shows that H2O2 has contrasting effects on viral replication depending on its concentration; it generates several antiproliferative responses, induces apoptosis, and inhibits viral replication and invasion.

Chronic inflammation has been proposed to play a central role in cancer development. Thus, inflammation is a potential target for lung cancer prevention and treatment. Inflammatory cells release a variety of cytokines, chemokines, cytotoxic mediators including ROS, metalloproteinases (MMPs), and membrane-perforating agents, and soluble mediators of cell death, such as TNF-$\alpha$ (Tumor Necrosis Factor-$\alpha$), interleukins (IL), and interferons (IFNs). Several important molecules involved in the inflammatory response are regulated by or have been associated with ROS and H2O2. (Vilema-Enríquez, 2016). The association of ROS with inflammation might be used to suggest combined treatments of H2O2 with anti-inflammatory drugs in COVID 19 therapy.

Recently, several drugs indicated for other diseases have been shown to have antiproliferative properties and have been suggested as an alternative therapy for different malignancies including COVID 19. Thus, the novel combination of H2O2 with such repositioned drugs represents a new research area in COVID 19 therapy.

Because of the multimechanistic and multitarget anticancerproperties of H2O2, this molecule is a very interesting potential therapeutic tool to fight COVID 19. The proper and cautious use of H2O2 in combination with commonly used drugs may have synergistic effects increasing viral inactivation. Particularly, novel therapeutic approaches combining H2O2 with repositioned drugs may help to decrease the mortality from this viral disease.

**Effects of Hydrogen Peroxide on Plasma Membrane and Calcium Mobilization**

Vilema-Enríquez (2016) studied the effects of hydrogen peroxide on plasma membrane and calcium mobilization. Ion channels play important roles in health and disease and Ca2+ signaling is an important second messenger that participates in many processes including proliferation and apoptosis. H2O2

increases intracellular Ca2+ concentration and decreases electrical resistance in human lung microvascular endothelial cells via activation of TRPV4 ion channels, through a mechanism that requires the Src tyrosine kinase Fyn. In addition, exposure to H2O2 increases intracellular Ca2+ concentration in rat alveolar type II epithelial cells and induces calcium release from the endoplasmic reticulum in endothelial cells.

Another plasma membrane effect of H2O2 is on adhesion molecules that are important for permeability and signaling transduction in lung epithelium. When A549 cells were stimulated byH2O2 the levels of the adhesion molecules CD49f, CD49b, CD29, and CD44 were increased. The expression of these molecules is closely associated with the stress response. The effect of H2O2 on the plasma membrane and intracellular calcium concentration may be already involved in triggering cell death Vilema-Enríquez (2016).

**Hydrogen Peroxide in Vascular Endothelial Cells**

A very important research on hydrogen peroxide signaling in vascular endothelial cells was carried out by Breton-Romero and Lamas (2014). They pointed out that endothelial cells (ECs) line the inner surface of the cardiovascular system acting as a natural barrier between the blood and the rest of organs and tissues. This metabolically active monolayer organ is constantly exposed to different biomechanical and biochemical stimuli to which it responds by preserving the integrity and homeostasis of vascular function. Accumulating evidence indicates the important role of redox signaling in the triggering and mediation of these actions. Historically, oxidative stress and thus, the increased production of reactive oxygen species (ROS), have been closely related with endothelial dysfunction, with involvement in the pathogenesis of several cardiovascular diseases such as hypertension, diabetes, or atherosclerosis, among others. However, a large body of research has demonstrated a key role for ROS as physiological regulators of intracellular signaling pathways involved in the function of vascular endothelium.

Among the different ROS, it is suggested by Breton-Romero and Lamas (2014) that hydrogen peroxide (H2O2) fulfills the prerequisites for serving as an intracellular messenger and acting as a cell-signaling molecule. H2O2 is a small and non-polar molecule able to diffuse across biological membranes. It is ubiquitously produced, and its longer half-life makes it suitable to act as a second messenger exerting prolonged effects in different signaling pathways. Intracellular generation of ROS in endothelial cells

both occur under physiological as well as pathophysiological conditions. In the endothelium it predominantly arises from four enzymatic systems which include the different isoforms of NAPDH oxidases, xanthine oxido-reductase, uncoupled endothelial nitric oxide synthase (eNOS) and mitochondrial respiration complexes.

All these sources primarily catalyze the reduction of molecular oxygen after the acceptance of one electron and lead to the formation of superoxide radical anion (O2 •−), a ROS extremely unstable that dismutates to H2O2 either spontaneously or enzymatically catalyzed by superoxide dismutase. Of note, some enzymes, such as glucose oxidase or xanthine oxidase have been described to directly produce H2O2 by donating two electrons to oxygen. In the case of the NOX4 isoform (the most abundant NADPH oxidase in the endothelium), there is some controversy about the ROS produced. Whereas some groups have described NOX4 as the only vascular homolog that directly produces H2 O2, others have proposed that the O 2 •−produced by NOX4 is rapidly converted to H2O2, which becomes almost undetectable (Breton-Romero and Lamas, 2014).

In vascular endothelial cells, ROS has gained attention as important second messengers by regulating the activity of signaling proteins, enzymes and ion channels in endothelial cells. H2O2 modulates different aspects of endothelial cell function, including endothelial cell growth and proliferation, survival, endothelium-dependent vasorelaxation, cytoskeletal reorganization, inflammatory responses and endothelium-regulated vascular remodeling, among others. Whereas a modest increase and a tight controlled regulation of H2O2 is essential for the maintenance of vascular homeostasis, an aberrant redox signaling, usually induced by an excessive production of ROS and / or by decreases in antioxidant activity, may contribute to an alteration in vascular function and lead to vascular disease (Darley-Usmar and White, 1997; Ramachandran et al, 2002 in Breton-Romero and Lamas, 2014).

In their research, Breton-Romero and Lamas (2014) concluded that Hydrogen peroxide acts as a signaling second messenger in the vasculature. Its targets in the cardiovascular system are diverse, and include different protein kinases, which convey a wide array of effects to the endothelium. Finally, signaling levels of H2O2 play a key role in vascular function and homeostasis.

The hydrogen peroxide regulation of endothelial function was studied by Cai (2005). The researcher established that H2O2 modulates different aspects of endothelial cell function including endothelial cell growth and proliferation, endothelial apoptosis, endothelium-dependent vasorelaxation, endothelial

cytoskeletal reorganization and barrier function, endothelial inflammatory responses, and endothelium-regulated vascular remodeling.

**Artemisia annua and Hydrogen Peroxide**

Poisson-Benatouil (2020), from the Maison de l'Artemisia, has explained that 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 A. annua, can also generate H2O2 via antioxidant iNO synthase enzymes. H2O2 can react with iron or copper to produce highly reactive OH radicals (Poisson-Benatouil, 2020).

In the liver, in addition to its cytotoxic effects, H2O2 plays an essential role as a molecule regulating the activation of the cellular immune defense. 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 (Poisson-Benatouil, 2020).

During inflammatory processes infiltrating cells produce large amounts of reactive oxygen intermediates (ROI). Increasing evidence suggests that ROI besides being cytotoxic may act as important mediators influencing various cellular and immunological processes. In this study, we have investigated the effects of hydrogen peroxide on several aspects of lymphocyte activation. In ESb-L T lymphoma cells, micromolar concentrations of hydrogen peroxide rapidly induced activation of the transcription factor NF-kappa B, whereas DNA-binding activity of the transcription factor AP-1 was virtually not affected. In addition, hydrogen peroxide induced early gene expression of interleukin-2 (IL-2) and the IL-2 receptor alpha chain. The stimulation of IL-2 expression was found to be conferred by a kappa B-like cis-regulatory region within the IL-2 gene promoter. In contrast to these activating effects, addition of hydrogen peroxide was largely inhibitory on cell proliferation which is consistent with

a general requirement of thiol compounds for lymphocyte proliferation. However, hydrogen peroxide significantly increased T cell proliferation when applied for a short period under reducing conditions. These data indicate that ROI may act as an important competence signal in T lymphocytes inducing early gene expression as well as cell proliferation (Los et al, 1995).

Hydrogen peroxide as second messenger in lymphocyte activation was studied by Reth (2002). It was determined that H2O2 also acts as an intracellular messenger in activated lymphocytes. Oxidants such as H2O2 are connected to lymphocyte activation, but the molecular mechanisms behind this phenomenon are less clear. Here, I review data suggesting that by inhibiting protein tyrosine phosphatases, H2O2 plays an important role as a secondary messenger in the initiation and amplification of signaling at the antigen receptor. These findings explain why exposure of lymphocytes to H2O2 can mimic the effect of antigen. In addition, more recent data show that antigen receptors themselves are H2O2-generating enzymes and that the oxidative burst in macrophages seems to play a role not only in pathogen killing but also in the activation of these as well as neighboring cells. Thus, by controlling the activity of the negative regulatory phosphatases inside the cell, H2O2 can set and influence critical thresholds for lymphocyte activation.

It has been observed by Mobili, et al (2013) that Artemisia annua infusions are only efficient in daylight, not in the dark. The UV/H2O2 system is well known as advanced oxidation process in which hydrogen peroxide is added in the presence of ultraviolet light to generate hydroxyl radicals. The oxidation potential of a hydroxyl radical is much greater than other oxidizing agents such as ozone and chlorine. Hydrogen peroxide strongly absorbs long wave present in daylight.

**Zinc Ionophores & Metal Chelation by Artemia annua**

Ionophores are compounds forming lipid-soluble complexes that act as vehicles for transporting ions across biological membranes. In their uncomplexed form they are straight-chain molecules, but they become cyclized by head-to-tail hydrogen bonding in the presence of appropriate cations Ionophores probably become arranged in a stack across the membrane, so forming a channel that is more or less selective for certain cations. Ionophores form charged complexes with cations which serve to transport the cations down their electrochemical gradient, which promote electrically neutral exchange diffusion of cations (Zn) across membranes. The ion selectivity of the ionophore is determined, at least partly,

by the structural constraints of the cavity through the stack of cyclized ionophore molecules (Bowman, 1990).

Ionophores are a class of compounds that form complexes with specific ions and facilitate their transport across cell membranes. An ionophore typically has a hydrophilic pocket (or hole) that forms a binding site specific for a particular ion (Freedman, 2012).

One major function of zinc to human is its ability to boost the body's immunity and fight viruses. Studies show that zinc can block the replication and growth of viruses in the body and in lab tests. In the body, zinc improves the actions of immune cells like Neutrophils, T cells, B cells and NK that act as the police of the body, which attack infections (Oyeniy, 2020).

Zinc is found and operates in every cell of the body, but it's a non-fat-soluble mineral that can't move through the fat-based cell membrane. Therefore, it needs help to cross the cell membrane from special transport systems. These systems include zinc ionophore and zinc binding-proteins. The zinc binding-proteins are located in all membranes of every cell of the body for efficient inflow and outflow of zinc in the cells. Ionophore is a fat-soluble substance that can transport non-fat soluble elements across the cell membrane. Zinc-ionophores are zinc transporters in and out of the cell and can increase the effects of zinc in the cell (Oyeniy, 2020).

Bazarbachi (2015) did an extensive doctorate research on the fact that dietary polyphenols display zinc ionophore activity and modulate zinc signaling. Several compounds can chelate or interact with zinc ions forming different complexes with new chemical and biological properties. Some of them bind zinc ions making them non available for the cell acting as sequestrants. If those complexes cross the cell membrane in an independent way of membrane channels or transporters and release the metal ions in the cytoplasmic environment, they would not only act as zinc chelators (sequestrants), but also as molecules which enhances the zinc transport within the cell, also known as zinc ionophores. Several synthetic molecules known to act as zinc ionophores molecules are able to modulate intracellular labile zinc concentrations, and some of those are being tested as good candidates to treat some viral processes.

Several studies have supported the development of zinc ionophores as a novel group of antiviral agents. Natural occurring compounds can also interact with zinc forming complexes. The medical

properties of naturally occurring compounds such as chromones, coumarins and particularly polyphenols have been well described from many years. It hasto be highlighted that organic compounds, such as phenols and flavonoids, exhibit ionophoric properties (Bazarbachi, 2015).

Phenols and flavonoids are natural substances, which are found in Artemisia annua are potent zinc-ionophores. They are known for its antimicrobial, antiviral and anticancer properties and they are regarded as the safest zinc-ionophores compared to other ionophores like hydroxychloroquine. The scientific evidence points out that that the complexes of phenolic natural occurring compounds with zinc ions are more effective than the non-complexed single molecules. This will change the course of drug research, because these new formations could successfully be used in a range of multiple diseases such as diabetes, neurodegenerative diseases, obesity, cancer, bacterial and viral infections (Bazarbachi, 2015), including COVID-19.

Additionally, those phenolic complexes in Artemisia annua may play an important role in the modulation of the intracellular zinc homeostasis, which is an essential factor that affects several biological functions, such as cell signaling.

Regarding the interaction with metals in the last decades, phenolic compounds have been known to interact with different metals, and because of their distinctive chemical structure, they can easily form complexes through metal ion chelation, which is thought to be the most important mechanism of flavonoids to exert their antioxidant activity beside with many other biological effects that can be altered by those interactions. The antioxidant activity results from binding metal ions like Fe(II), Fe(III), Cu(I) or Zn(II) which participate in free radical-generating reactions. Therefore, they act on two antioxidant pathways; on one hand there is a direct reaction with free radicals and on the other hand the chelation of metal ions involved in production of reactive oxygen species (Bazarbachi, 2015).

Experimental data indicated that the chelated compounds were more effective free radical scavengers than flavonoids alone. These results suggest that the metal-flavonoid complexes, not only exert singular biological properties, but also can enhance the effects of both compounds individually. One of the mechanisms by which flavonoids exert their antioxidant activity is by chelating redox-active transition metals, mainly zinc, iron and copper, which are known to catalyze many biological processes leading to the production of free radicals. The essential sites for metal chelation are hydroxyl groups, and the most suitable cations for chelation are Fe(II), Fe(III), Cu(II) and Zn(II) because of their high

charge density, stimulating the interaction with the phenoxide groups which have a high negative charge density (Bazarbachi, 2015).

Some of the formed complexes could exert many other biological effects, such as molecular signaling, particularly if the metal ion exerts that effect by itself. For example, the interaction of flavonoids with zinc ions could enhance the effect of both molecules lonely. Several studies have revealed that polyphenols not only interact with metal ions, but they deeply modulate expression of MTs, cellular zinc transporters, extracellular zinc carriers, and intracellular zinc accumulation which are key factors in zinc homeostasis. In addition, an increase of Zinquin (fluorescent specific zinc indicator)-detectable cytoplasmic levels of zinc in HepG2 cell line were monitored when treated with phenolic compounds. This increment in intracellular zinc levels have been reported to induce apoptosis of tumour cells (Bazarbachi, 2015).

Quercetin, which is present in Artemisia annua, has shown the deepest metal interaction, probably because of its ability to assume a planar conformation. At physiologic pH, most polyphenols interact with the polar head groups of phospholipids at the membrane surface via the formation of hydrogen bonds that involve the hydroxyl groups of the polyphenols. A high number of hydroxyl groups on the polyphenol structure and an increase in pH that leads to deprotonation of the hydroxyl groups would thus enhance interactions between the polyphenols and the membrane surface (Bazarbachi, 2015).

The complexation of metal ions to flavonoids gives a specific spatial orientation, and this could be one of the reasons for the pharmacological activity of Artemisia annua. These metal ion complexes therefore can exhibit similar characteristics as their parent flavonoids and also display unique features distinct from their parent flavonoid due to their structural characteristics. Several recent reports have indicated that the flavonoid–metal ion complexes possess more potent biological activities than the parent. Many pharmacological effects have been identified for flavonoid-metal ion complexes, such as: antiviral, anti-bacterial, anti-inflammatory, antioxidant, anti-tumor, etc. (Bazarbachi, 2015).

Zinc, the second most abundant transition metal in humans, is an essential micronutrient with structural, catalytic and signaling function and shows antioxidant action in cells. Zinc is found in high concentrations in Artemisia annua. Quercetin is one of the most abundant flavonoids found in Artemisia annua, which plays a relevant role in health benefits, in part, based on its antioxidant actions, which partially derives from its interaction with the redox-active transition metals iron, copper and zinc. The

interaction of Quercetin with the redox-inert zinc and the biological effects of this interaction have been barely studied. Polyphenols are known to act as antioxidants and as signaling molecules, and to form complexes with Zinc, copper and iron thereby impacting their bioavailability.

Bazarbachi (2015) carried out a research study to determine whether Quercetin chelates zinc cations affects zinc homeostasis. In this work, we seek to check whether Quercetin interacts with zinc and to evaluate the effect of this interaction on intracellular zinc homeostasis and zinc signaling in an in vitro model. It was observed that Quercetin strongly binds to zinc cations in solution as shown by the quenching of zinc dependent Zinquin fluorescence. Administration of Quercetin together with supplemental zinc results in enhanced expression of the zinc-store protein metallothionein and the zinc-export transporter ZnT1. Total intracellular zinc content and cytoplasmic labile zinc (monitored by Zinquin fluorescence) are increased. Combinations of zinc with Quercetin are more effective that the single compounds in modulating intracellular zinc homeostasis and signaling. It was found out that Quercetin acts as zinc ionophore increasing intracellular total zinc levels and it chelates zinc cations in solution. In addition, Quercetin elevates cytoplasmic labile zinc.

Quercetin forms membrane-permeable complexes with zinc, which then acts as ionophores and therefore transport the metal into the cell. Once within the cell, Quercetin is metabolized and the zinc cations are added to the labile pool of zinc. Flavonoids enhance cytoplasmic labile zinc and the consequences of this enhancement have an impact on the modulation of zinc signaling and metabolic pathways (Bazarbachi, 2015).

**Combination Therapies**

Rayner et al (2020) in their publication “Accelerating Clinical Evaluation of Repurposed Combination Therapies for COVID-19” have explained that as the global COVID-19 pandemic continues, unabated and clinical trials demonstrate limited effective pharmaceutical interventions, there is a pressing need to accelerate treatment evaluations. Among options for accelerated development is the evaluation of drug combinations in the absence of prior monotherapy data. This approach is appealing for a number of reasons. First, combining two or more drugs with related or complementary therapeutic effects permits a multipronged approach addressing the variable pathways of the disease. Second, if an individual component of a combination offers a therapeutic effect, then in the absence of antagonism, a trial of combination therapy should still detect individual efficacy. Third, this strategy is time saving.

Rather than taking a stepwise approach to evaluating monotherapies, this strategy begins with testing all relevant therapeutic options. Finally, given the severity of the current pandemic and the absence of treatment options, the likelihood of detecting a treatment effect with combination therapy maintains scientific enthusiasm for evaluating repurposed treatments. Antiviral combination selection can be facilitated by insights regarding SARS-CoV-2 pathophysiology and cell cycle dynamics, supported by infectious disease and clinical pharmacology expert advice. Rayner et al (2020) described a clinical evaluation strategy using adaptive combination platform trials to rapidly test combination therapies to treat COVID-19.

Artemisia annua + Zinc for the Treatment of COVID-19 may offer a potential successful combination therapy with Ivermectin.

**Ivermectin**

**Ivermectin and Viral Inhibition**

On April 3, 2020, a famous scientific publication by Caly et al (2020) was available online. It reported that ivermectin, an FDA-approved anti-parasitic, previously shown to have broad-spectrum anti-viral activity in vitro, is an inhibitor of the causative virus, SARS-CoV-2. A single addition of ivermectin to Vero-hSLAM cells, 2 hours post infection with SARS-CoV-2, was able to effect ~5000-fold reduction in viral RNA at 48 h.

Since that publication of Caly et al (2020), an overwhelming amount of scientific studies and testimonies around the entire globe have been reported about the efficacy of ivermectin. Here, a representative report by Formiga et al (2020) of some of the work being done in Brazil is presented:

In light of the potential of ivermectin to prevent replication in a broad spectrum of viruses, Rocha Formiga et al (2020) have discussed the inhibition of importin α/β1-mediated nuclear import of viral proteins as the probable mechanism underlying its antiviral activity. Since SARS-CoV-2 is an RNA virus, a similar mechanism of action may take place. A possible ionophore role for ivermectin has also been reported. Since ionophore molecules have been described as potential antiviral drugs, ivermectin could ultimately induce an ionic imbalance that disrupts the potential of the viral membrane, thereby threatening its integrity and functionality.

Formiga et al (2020) have illustrated that the pathology of COVID-19 is characterized by the rapid replication of SARS-CoV-2, triggering an amplified immune response that may lead to cytokine storm, which frequently induces a severe inflammatory pulmonary response. Disease progression may result in progressive respiratory failure arising from alveolar damage and can lead to death. Moreover, the monitoring of SARS-CoV-2 viral load in the upper respiratory tract and bronchoalveolar lavage fluid (BALF) in patients with severe disease indicates higher loads, as well as greater viral persistence.

Regarding its anti-inflammatory properties, ivermectin has been shown to mitigate skin inflammation. Importantly, ivermectin significantly diminished the recruitment of immune cells and cytokine production in BALF assessed in a murine model of asthma. A study evaluating the ability of ivermectin to inhibit lipopolysaccharide (LPS)-induced inflammation revealed significantly decreased production of TNF-alpha, IL-1ss and IL-6 in vivo and in vitro. Further studies may establish the role of ivermectin in the inflammatory response caused by SARS-CoV-2. Besides the antiviral activity ivermectin could play a supportive adjuvant role facing the hostile infection microenvironment (Rocha Formiga et al, 2020).

**Ivermectin Mode of Action**

Gupta, Sahoo and Singh (2020) have reported that recently, the antiviral function of ivermectin has been discovered, which appears to be intriguing. Already its effectiveness against certain flavivirus (dengue fever, Japanese encephalitis and tick-borne encephalitis virus) and chikungunya virus has been demonstrated in vitro. Since then, the same activity has been assessed in numerous other viral infections. Currently, its potency has been recognized in eliminating coronavirus in vitro. The exact mechanism to which this effect can be attributed is yet to be validated. The speculated mechanism is inhibition of importin α/β1 mediated transport of viral proteins in and out of the nucleus. Importins, types of karyopherins, exemplify a major class of soluble transport receptors which are involved in nucleo-cytoplasmic transit of various substrates. The speculated inhibitory action of ivermectin on importin α/β1 mediated transport system explains the role of ivermectin in eliminating Covid-19 (Mastrangelo et al, 2012, Varghese et al , 2016, Tessier et al, 2019, Oka &Yoneda, 2018, Gupta, Sahoo and Singh, 2020).

In a single in vitro study, the efficacy of ivermectin against coronavirus was demonstrated by Caly et al. (2020). The in vitro potency of ivermectin against Covid-19 virus is evidence that this drug can be utilized to manage patients infected with SARS-CoV-2. Upon comparison, Ivermectin may prove to

have leverage over the other pharmacotherapeutic options for the management of Covid-19. Furthermore, the treatment regimen with Ivermectin is very cost-effective. Considering these merits, it becomes imperative that multiple clinical trials with Ivermectin be conducted (Gupta, Sahoo and Singh, 2020).

Kory et al (2021) reported that in March 2020, the Front Line COVID-19 Critical Care Alliance (FLCCC) was created and led by Professor Paul E. Marik to continuously review the rapidly emerging basic science, translational, and clinical data to develop a treatment protocol for COVID-19. The FLCCC then recently discovered that ivermectin, an anti-parasitic medicine, has highly potent anti-viral and anti-inflammatory properties against COVID-19. They then identified repeated, consistent, large magnitude improvements in clinical outcomes in multiple, large, randomized and observational controlled trials in both prophylaxis and treatment of COVID-19. Further, data showing impacts on population wide health outcomes have resulted from multiple, large "natural experiments" that occurred when various city mayors and regional health ministries within South American countries initiated "ivermectin distribution" campaigns to their citizen populations in the hopes the drug would prove effective. The tight, reproducible, temporally associated decreases in case counts and case fatality rates in each of those regions compared to nearby regions without such campaigns, suggest that ivermectin may prove to be a global solution to the pandemic. This was further evidenced by the recent incorporation of ivermectin as a prophylaxis and treatment agent for COVID-19 in the national treatment guidelines of Belize, Macedonia, and the state of Uttar Pradesh in Northern India, populated by 210 million people. For Kory et al (2021), their review is the earliest to compile sufficient clinical data to demonstrate the strong signal of therapeutic efficacy as it is based on numerous clinical trials in multiple disease phases. One limitation is that half the controlled trials have been published in peer-reviewed publications, with the remainder taken from manuscripts uploaded to medicine pre-print servers. Although it is now standard practice for trials data from pre-print servers to immediately influence therapeutic practices during the pandemic, given the controversial therapeutics adopted as a result of this practice, the FLCCC argues that it is imperative that our major national and international health care agencies devote the necessary resources to more quickly validate these studies and confirm the major, positive epidemiological impacts that have been recorded when ivermectin is widely distributed among populations with a high incidence of COVID-19 infections.

## Conclusions

Artemisia annua as a potential Source of Molecules with Pharmacological Activity in Human Diseases was studied by Mesa et al (2015). Their review intended to motivate and encourage researchers to explore new alternatives to treat different diseases with Artemisia annua, an important plant of traditional Chinese pharmacopoeia that has been used for more than 2.000 years in the treatment of different diseases, mainly malaria. Their review highlighted the pharmacological potential of the A. annua plant in the treatment of several infectious diseases and unveils its suitable profile of safety and tolerability.

In Mexico City, The Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran(2020) is carrying out a randomized, double-blind, placebo-controlled, multi-arm, multicenter, phase II trial design to allow a rapid efficacy and toxicity assessment of potential therapies, camostat mesilate (serine protease inhibitor) and Artemisia annua (unknown mechanism) immediately after COVID-19 positive testing in mild to moderate disease and high-risk factors such as diabetes, hypertension, and obesity among others. The hypothesis of this study is that the addition of agents that inhibit viral entry or replication of SARS-CoV-2 virus, such as Artemisia annua and camostat, will reduce the rate of a composite outcome of hospitalization due to COVID-19 pneumonia or the use of oxygen therapy; will be devoid of additional moderate to severe toxicities; and will improve viral clearance at Day 14 in high-risk individuals. The main hypothesis is that the clinical outcomes in COVID-19 infected patients at higher risk of poor outcomes following infection will be improved compared to the standard of care when introduced as an early intervention after diagnosis.

**Intervention:** (1) Camostat mesylate. 100 mg tablet, 600 mg/day. Oral, 2 tablets three times a day, after a meal (600 mg total daily dose) Days 1-14. (2) Artemisia annua Tea 225mg per bag,1350 mg/day. Oral, one 8 oz brewed tea (two bags) three times a day, Days 1-14.

In a Clinical trial The Princess Nourah Bint Abdulrahman University, in Saudi Arabia, their investigators are evaluating the effect of Artemisinin / Artesunate on morbidity of COVID-19 patients in decreasing the course of the disease and viral load in symptomatic stable positive swab COVID-19 patients. Investigators are hypothesizing that due to the antiviral properties of this drug it will help as a treatment for the COVID -19 patients. In improving their condition and clearing the virus load.

**Intervention:** Artemisinin / Artesunate 100mg once daily for 5 days.

The Mahatma Gandhi Mission Medical College and Hospital (India), Hillel Yaffe Medical Center (Israel), Nazareth Hospital EMMS (Israel) and Rambam Health Care Campus (Israel) (2020), will conduct a Phase II, Controlled Clinical Study Designed to Evaluate the Effect of ArtemiC in Patients Diagnosed With COVID-19. ArtemiC is a medical spray comprised of Artemisinin (6 mg/ml), Curcumin (20 mg/ml), Frankincense (=Boswellia) (15 mg/ml) and vitamin C (60 mg/ml) in micellar formulation for spray administration. Patients will receive up to 6 mg Artemisinin, 20 mg Curcumin, 15 mg Frankincense and 60 mg vitamin C given daily as an add-on therapy (in addition to standard care) in two divided doses, on Days 1 and 2. Patient follow-up will last 2 weeks. During this time, patients will be monitored for adverse events. Overall rationale A preparation of ArtemiC, comprising Artemisinin, Curcumin, Boswellia, and Vitamin C in a nanoparticular formulation, is proposed as a treatment for the disease associated with the novel corona virus SARS-CoV-2. It is readily available in light of its status as a food supplement. This initiative is presented under the urgent circumstances of the fulminant pandemic caused by this lethal disease, which is known as COVID-19 and has spread across the globe causing death and disrupting the normal function of modern society. The grounds for the proposal are rooted in existing knowledge on the components and pharmacological features of this formulation and their relevance to the current understanding of the disease process being addressed.

Leading among these considerations are well established immuno-modulatory activities of the active ingredients as established in vitro and in vivo and published over the years. These activities as apparent, for example, in diminishing activity of TNF alpha and IL-6 levels are acknowledged to be relevant to the pathophysiology processes involved in the progressive form of COVID-19. The active agents have in addition prominent anti-oxidant, anti-inflammatory as well as anti-aggregant and anti-microbial activities.

**Intervention:** Treatment will be sprayed orally twice a day for the first 2 days in the treatment period.

A multi-center, stage 2, randomized, Phase 2/3 study to evaluate the safety and efficacy of pyronaridine-artesunate in participants with corona virus disease 2019 (COVID-19) is carried out by The Philippine General Hospital.

**Intervention:**

The Drug: Artecom® (pyronaridine-artesunate). ≥ 65kg (Artecom® 4 tablets: Pyronaridine 720mg/ Artesunate 240mg). ≥ 45kg and < 65kg (Artecom® 3 tablets: Pyronaridine 540mg/ Artesunate 180mg). Artecom® is treated orally once a day for 3 consecutive days.

Korea University Ansan Hospital et al (2020) designed a clinical trial to establish the efficacy and safety of Pyramax in mild to moderate COVID-19 Patients.

**Intervention:** Pyramax (Pyronaridine 180mg/ Artesunate 60mg), with time frame: Day 3, 7, 10, 14.

In another clinical trial the University of Kentucky (2020) study novel agents for treatment of high-risk COVID-19 positive patients. This is a multi-arm, phase II trial for rapid efficacy and toxicity assessment of multiple therapies immediately after COVID19 positive testing in high-risk individuals. Therapies include stand-alone or combination treatment with hydroxychloroquine, azithromycin, ivermectin, or camostat mesilate, artemesia annua. The hypothesis of this study is that the addition of agents that inhibit viral entry or replication of SARS-CoV-2 virus replication in will be devoid of additional moderate to severe toxicities, will prevent clinical deterioration, and will improve viral clearance in high-risk individuals.

**Intervention**

Ivermectin: Days 1-2: Weight < 75kg: 4 tabs (12 mg total daily dose) Days 1-2: Weight > 75kg: 5 tabs (15 mg total daily dose). Camostat Mesilate: Days 1-14: 2 tab TID after a meal (600 mg total daily dose). Artemesia annua: Days 1-14: tea or coffee pod TID (1350 mg total daily dose). Artesunate: Days 1-14. Time Frame of 40 days

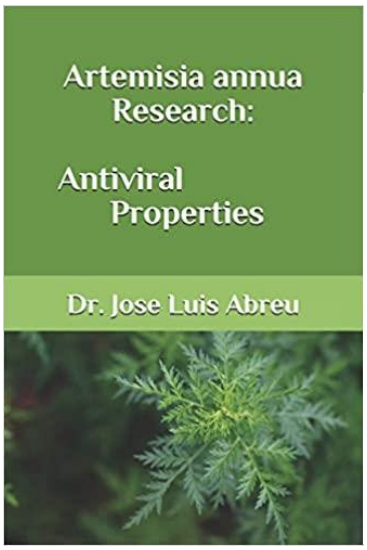
In a letter to the editor of Integrative Medicine Research, Leung and Xu (2020) exposed that artemisinin is the most important bioactive component in Artemisia annua. The chemical structure of artemisinin is a sesquiterpene lactone containing an unusual endoperoxide bridge with chemical formulas C15H22O5and a molecular weight of282.332 g/mol. This unusual endoperoxide bridge is the key active site for its drug mechanism of action. However, artemisinin has certain limitations such as poor water insolubility and bioavailability, so various semi-synthetic derivatives developed including

dihydroartemisinin, B-artemether and artesunate which exhibit greater potency, improve the water solubility, favorable metabolic as well as the hydrolytic stabilities.

In 2005, Li et al. have been indicated that artemisinin is one of the candidates to treat severe acute respiratory syndrome coronavirus (SARS-CoV) in Vero cell-based CPE/MTS screening. It's inhibited the coronavirus replication and showed antiviral activity against SARS-CoV with 34.5 ± 2.6 μg/mL in 50% effect concentration (EC50), CC50value of 1053.0 ± 92.8 μg/mL in cyto-toxicity assay and a selective index (SI) is greater than 31 which shown that artemisinin could be further developed as a drug for coronavirus.

Traditional Chinese herb "Artemisia annua" may be a good choice as the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is extremely similar to the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003. The pharmacological mechanism of Artemisia annua has mainly inhibited the enzymatic activity of CLPro (chymotrypsin-like protease) which is an enzyme produced by SARS-CoV-2 during COVID-19 infection. It is expected to be improved exhausting adaptive immunity and modulating the inflammatory response through regulating the production of pro-inflammatory cytokines such as prostaglandin E2 (PGE2), IL-6, IL-10 and TNF alpha. The genesis of CD4, CD8 and interferon-gamma would be increased when a combination of the minerals and biomolecules. The principle is the same as artemisinin-based combination therapies (ACTs) and well developed previously in 2003 (Leung and Xu, 2020).

In the letter, Leung and Xu (2020) concluded that the traditional Chinese herb "Artemisia annua" very likely combats COVID-19. However, more work needs to be done for supporting the evidence including its safety and efficacy to fight against COVID-19.




amazon

**Purchase**

---

## References

A. Ramachandran, A.L. Levonen, P.S. Brookes, E. Ceaser, S. Shiva, M.C. Barone, et al, Mitochondria, nitric oxide, and cardiovascular dysfunction, Free Radical Biology & Medicine. 33 (2002) 1465–74. http://dx.doi.org/10.1016/ S0891-5849(02)01142- 5 , 12446203 .

Albert Wingnang Leung and Chuanshan Xu. 2020. Is the traditional Chinese herb “Artemisia annua” possible to fight against COVID-19?. Integrative Medicine Research 9 (2020) 100474.

Amit Kumar, Yuichi Kubota, Mikhail Chernov, Hidetoshi Kasuya. 2020. Potential role of zinc supplementation in prophylaxis and treatment of COVID-19. Medical Hypotheses Volume 144, November 2020.

Arturo A. Caruso MD, Antonio Del Prete MD, Antonio I. Lazzarino PhD, Roberto Capaldi MD and Lucia Grumetto. 2020. Letter to the Editor. Might hydrogen peroxide reduce the hospitalization rate and complications of SARS-CoV-2 infection? Infection Control & Hospital Epidemiology (2020), 1–2.

B. Ivanescu, L. Vlase, A. Corciova, and M. I. Lazar. 2010. HPLC-DAD-MS STUDY OF POLYPHENOLS FROM Artemisia absinthium, A. annua, and A. vulgaris. Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

Camelia Paula Ştefanache, Oana-Crina Bujor, Radu Necula, Doina Dănilă, Nina Ciocârlan, Veaceslav Ghendov, Christoph Carlen, Xavier Simonnet. 2016. Phenolic Content of Artemisia annua L. From Natural Habitats in Republic of Moldova. J. Plant Develop. 23(2016): 61-69

Carmen M. Donangelo, Leslie R. Woodhouse, Sarah M. King, Fernando E. Viteri and Janet C. King. 2002. Supplemental Zinc Lowers Measures of Iron Status in Young Women with Low Iron Reserves. J. Nutr. 132: 1860–1864, 2002.

Catherine Poisson-Benatouil. 2020. Action of Artemisia annua on adaptive immunity in COVID-19 infections. Concept note. Maison de l’Artemisia Pointe-Noire | Republic of Congo.

Christian M. Wallen, John Bacsa and Christopher C. Scarborough. 2015. Hydrogen Peroxide Complex of Zinc. J. Am. Chem. Soc. 2015, 137, 46, 14606–14609

Craig R. Rayner, Louis Dron, Jay J. H. Park, Eric H. Decloedt, Mark F. Cotton, Vis Niranjan, Patrick F. Smith, Michael G. Dodds, Fran Brown, Gilmar Reis, David Wesche, and Edward J. Mills. 2020. Accelerating Clinical Evaluation of Repurposed Combination Therapies for COVID-19. Am. J. Trop. Med. Hyg., 103(4), 2020, pp. 1364–1366

Eby G. A. 1997. Zinc ion availability—the determinant of efficacy in zinc lozenge treatment of common colds. J Antimicrob Chemother 1997;40(4):483–93.

Elaine M Aldred. 2009. Pharmacology: A Handbook for Complementary Healthcare Professionals. Churchill Livingstone

Fabio Rocha Formiga, Roger Leblanc, Juliana de Souza Rebouças, Leonardo Paiva Farias, Ronaldo Nascimento de Oliveira, Lindomar Pena. 2020. J Control Release. 2020 Oct 7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7539925/

Gabriela Vilema-Enríquez, Aurora Arroyo, Marcelo Grijalva, Ricardo Israel Amador-Zafra, and Javier Camacho. 2016. Oxidative Medicine and Cellular Longevity Volume 2016, Article ID 1908164, 12 pages.

Gupta, D. et al. Ivermectin: potential candidate for the treatment of COVID19. Braz J Infect Dis. 2020.

H. A. Blas López, G. H. Lopes, A. C. Silva Costa Teixeira, C. E. Flores Barreda, P. A. Pantoja. 2017. Optimal Conditions for Oxidizing Phenol in Water by Fenton and Photo-Fenton Processes. Rev Soc Quím Perú. 83(4) 2017

H. Zhao, F. Granberg, L. Elfineh et al. (2003). Stategic attack on host cell gene expression during adenovirus infection, J Virol. 2003 Oct; 77(20): 11006–11015.

Hadi Ghaffari, Ahmad Tavakoli, Abdolvahab Moradi, Alijan Tabarraei, Farah Bokharaei-Salim, Masoumeh Zahmatkeshan, Mohammad Farahmand, Davod Javanmard, Seyed Jalal Kiani, Maryam Esghaei, Vahid Pirhajati-Mahabadi, Seyed Hamidreza Monavari and Angila Ataei-Pirkooh. 2019. Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: another emerging application of nanomedicine. Journal of Biomedical Science (2019) 26:70

Hamilton, G. A.; Friedman, J. P.; Campbell, P. M.; J. Am. Chem. Soc. 1966a, 88, 5266.

Helmut Sies. 2017. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biology 11 (2017) 613–619

https://malariaworld.org/blog/do-artemisia-annua-and-artemisinin-have-hormetic-effect

https://malariaworld.org/blog/hydrogen-peroxide-and-its-hidden-power

Hua Cai. 2005. Hydrogen peroxide regulation of endothelial function: Origins, mechanisms, and consequences. Cardiovascular Research 68 (2005) 26 – 36.

Husam Dabbagh Bazarbachi. 2015. Dietary Polyphenols Display Zinc Ionophore Activity and Modulate Zinc Signaling in Hepatocarcinoma Cells. Doctoral Thesis. Universitat Rovira I Virgili. Tarragona, Spain.

Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran. 2020. Camostat and Artemisia Annua vs Placebo in COVID-19 Outpatients. https://clinicaltrials.gov/

J. M. Phillips, T. Gallagher, Susan R. Weiss. 2017. Neurovirulent murine coronavirus JHM.SD use cellular zinc metalloproteases for virus entry and cell-cell fusion, Journal of Virology, 91, issue 8 (2017) 1-20.

Jeffrey C. Freedman. 2012. Ionophores in Planar Lipid Bilayers. Cell Physiology Source Book (Fourth Edition), 2012

Jinhuan Jiang, Jiang Pi, and Jiye Cai. 2018. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. Bioinorganic Chemistry and Applications. Volume 2018.

Jorge F.S. Ferreira, Devanand L. Luthria, Tomikazu Sasaki and Arne Heyerick. 2010. Flavonoids from Artemisia annua L. as Antioxidants and Their Potential Synergism with Artemisinin against Malaria and Cancer. Molecules 2010, 15, 3135-3170

Jun-Young Yang, Jiyeong Bae, Ayoung Jung, Seonyeong Park, Seungtae Chung, Jihyun Seok, Hangsik Roh, Youngju Han, Jae-Min Oh, Soojung Sohn, Jayoung Jeong, Wan-Seob Cho. 2017. Surface functionalizationspecific binding of coagulation factors by zinc oxide nanoparticles delays coagulation time and reduces thrombin generation potential in vitro. PLoS ONE 12(7): e0181634. https://doi.org/ 10.1371/journal.pone.0181634

K. Briknarova, C.J. Thomas, J. York and J.H. Nunberg. 2011. Structure of a zinc-binding domain in the Junin Virus envelope glycoprotein, Journal of Biological Chemistry, 286, No. 2 (2011) 1528-1536.

Korea University Ansan Hospital et al. 2020. The Efficacy and Safety of Pyramax in Mild to Moderate COVID-19 Patients. https://clinicaltrials.gov/

Leidi Cecilia Friedrich, Maria Anita Mendes, Volnir Oliveira Silva, Carmem Lúcia P. S. Zanta, Amilcar Machulek Jr. and Frank Herbert Quina. 2012. Mechanistic Implications of Zinc(II) Ions on the Degradation of Phenol by the Fenton Reaction.

Leon Calya, Julian D. Drucea, Mike G. Cattona, David A. Jansb, Kylie M. Wagstaffb. 2020. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Research. Volume 178, June 2020, 104787

Li S, Chen C, Zhang H, Guo H, Wang H, Wang L, et al. Identification of naturalcompounds with antiviral activities against SARS-associated coronavirus. AntivirRes 2005;67:18–23.

Los M, Dröge W, Stricker K, Baeuerle PA, Schulze- Osthoff K. 1995. Hydrogen peroxide as a potent activator of T lymphocyte functions. European Journal of Immunology. 1995 Jan; 25 (1): 1596 165.

Luz Estella Mesa, Pierre Lutgen, Ivan D. Velez, Angela M. Segura and Sara M. Robledo. 2015. Artemisia annua L., Potential Source of Molecules with Pharmacological Activity in Human Diseases. AJPCT[3][05][2015] 436-450

Manuel Olivares, Fernando Pizarro, Diego Gaitan, Manuel Ruz. 2007. Acute inhibition of iron absorption by zinc. Institute of Nutrition and Food Technology (INTA), University of Chile

Michael Reth. 2002. Hydrogen peroxide as second messenger in lymphocyte activation. Nat Immunol. 2002 Dec ; 3 (12): 1129 – 34.

Naira Josele Neves de Brito, Érika Dantas de Medeiros Rocha, Alfredo de Araújo Silva, João Batista Sousa Costa, Mardone Cavalcante França, Maria das Graças Almeida, José Brandão-Neto. 2014. Oral Zinc Supplementation Decreases the Serum Iron Concentration in Healthy Schoolchildren: A Pilot Study. Nutrients 2014, 6, 3460-3473; doi:10.3390/nu6093460

Niaz Shahed Ahmed. 2019. Zinc is an Intracellular Secondary Messenger in Platelets. Doctorate Thesis at Anglia Ruskin University. Department of Biomedical and Forensic Sciences.

Olga B. Mobili, Moïse Nola, Mireille E. Nougang, Luciane M. Moungang, Olive V. Noah Ewoti, Henriette Ateba Bessa and Joseph Mabingui. 2013. Assessment of the effect of Artemisia annua leave extract infusion pH under dark conditions on Staphylococcus aureus, Salmonella paratyphi and Escherichia coli. 2013. Journal of Applied Biosciences 62: 4595 – 4609

Oyeniy, S. O. 2020. Studies Show Zinc Inhibits Viral Replication, but There's a Catch. https://www.prnewswire.com/news-releases/studies-show-zinc-inhibits-viral-replication-but-theres-a-catch-301079772.html

Pamela J Weathers, Melissa Towler, Ahmed Hassanali, Pierre Lutgen, Patrick Ogwang Engeu. 2014. Dried-leaf Artemisia annua: A practical malaria therapeutic for developing countries?. World J Pharmacol 2014 December 9; 3(4): 39-55

Pierre Kory, MD, G. Umberto Meduri, MD, Jose Iglesias, DO, Joseph Varon, MD, Keith Berkowitz, MD, Howard Kornfeld, MD, Eivind Vinjevoll, MD, Scott Mitchell, MBChB, Fred Wagshul, MD, Paul E. Marik, MD. 2021. Review of the Emerging Evidence Demonstrating the Efficacy of Ivermectin in the Prophylaxis and Treatment of COVID-19. FLCCC.

Princess Nourah Bint Abdulrahman University. 2020. Evaluating the Efficacy of Artesunate in Adults with Mild Symptoms of COVID-19. https://clinicaltrials.gov/

R. Kumar, G. Sahoo, K. Pandey, M.K. Nayak, R. Topno, V. Rabidas, P. Das. 2018. Virostatic potential of zinc oxide (ZnO) nanoparticles on capsid protein of cytoplasmic side of chikungunya virus. Abstracts / International Journal of Infectious Diseases 73S (2018) 3–398

Rafah Hasan, Lothar Rink and Hajo Haase. 2012. Zinc signals in neutrophil granulocytes are required for the formation of neutrophil extracellular traps. Innate Immunity 19(3) 253–264

Rosa Breton-Romero and Santiago Lamas. 2014. Hydrogen peroxide signaling in vascular endothelial cells. Redox Biology 2 (2014) 529–534

Schoggins J. W, Wilson S. J, Panis M, Murphy M. Y, Jones C. T, Bieniasz P, Rice C. M. 2011. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 2011; 472(7344):481–5.

Scott A Read; Stephanie Obeid; Chantelle Ahlenstiel and Golo Ahlenstiel. 2019. Adv Nutr 2019;10:696–710.

Tang, F. S. M., Van Ly, D., Spann, K., Reading, P. C., Burgess, J. K., Hartl, D., Baines, K. J., & Oliver, B. G. (2016). Differential neutrophil activation in viral infections: Enhanced TLR-7/8-mediated CXCL8 release in asthma. Respirology, 21(1), 172-179. https://doi.org/10.1111/resp.12657.

te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, et al. (2010) Zn2+ Inhibits Coronavirus and Arterivirus RNA Polymerase Activity In Vitro and Zinc Ionophores Block the Replication of These Viruses in Cell Culture. PLoS Pathog 6(11): e1001176. doi:10.1371/journal. ppat.1001176

Tellinghuisen T. L, Marcotrigiano J, Gorbalenya A. E, Rice C. M. 2004. The NS5A protein of hepatitis C virus is a zinc metalloprotein. J Biol Chem 2004; 279(47):48576–87.

The Mahatma Gandhi Mission Medical College and Hospital et al. 2020. Phase II, Controlled Clinical Study Designed to Evaluate the Effect of ArtemiC in Patients Diagnosed With COVID-19. https://clinicaltrials.gov/

The Philippine General Hospital. 2020. The Safety and Efficacy of Pyronaridine-artesunate (Pyramax® or Artecom®)in COVID-19 Patients (PROVIDENCE). https://clinicaltrials.gov/

Tsuneo Ishida. 2018. Antiviral Activities of Zn2+ Ions for Viral Prevention, Replication, Capsid Protein in Intracellular Proliferation of Viruses. WSN 97 (2018) 28-50.

Tsuneo Ishida. 2019. Anti-Viral Vaccine Activity of Zinc(Ⅱ) for Viral Prevention, Entry, Replication, and Spreading During Pathogenesis Process. Curr Trends Biomedical Eng & Biosci. Volume 19 Issue 3 - July 2019

University of Kentucky. 2020. Novel Agents for Treatment of High-risk COVID-19 Positive Patients. https://clinicaltrials.gov/

V. Darley-Usmar, R. White, Disruption of vascular signalling by the reaction of nitric oxide with superoxide: implications for cardiovascular disease, Experimental Physiology. 82 (1997) 305–16

Vicky C. Roa-Linares, Yaneth Miranda-Brand, Verónica Tangarife-Castaño, Rodrigo Ochoa, Pablo A. García, Mª Ángeles Castro, Liliana Betancur-Galvis, and Arturo San Feliciano. 2019. Anti-Herpetic, Anti-Dengue and Antineoplastic Activities of Simple and Heterocycle-Fused Derivatives of Terpenyl-1,4-Naphthoquinone and 1,4-Anthraquinone. Molecules 2019, 24, 1279; doi:10.3390/molecules24071279

Wan-Fei Li, Wai I. Chik, Dong-Ying Wang, Lu-Tai Pan. 2017. Plant Phenolic Compounds as Potential Lead Compounds in Functional Foods for Antiviral Drug Discovery. Current Organic Chemistry Volume 21, Issue 18, 2017.

William C. Bowman. 1990. Pharmacology of Neuromuscular Function (Second Edition), 1990

Xinliang Liu et al. 2018. Progress in the Mechanism and Kinetics of Fenton Reaction. MOJ Ecology & Environmental Science. Volume 3 Issue 1 – 2018.

Yi Song, Kebede Taye Desta, Gon-Sup Kim, Soo Jung Lee, Won Sup Lee, Yun-Hi Kim, Jong Sung Jin, A. M. Abd El-Aty, Ho-Chul Shin, Jae-Han Shim and Sung Chul Shin. 2015. Polyphenolic profile and antioxidant effects of various parts of Artemisia annua L. Biochemical Chromatography, Volume30, Issue4.

## ABOUT THE AUTHOR

Dr. Jose Luis Abreu is an independent researcher in the field of Phytochemistry. He is also a Research-Professor and President of Spenta University Mexico (Monterrey, Mexico).

This study was funded with independent resources. There is not conflict of interest in this publication.

Email: spentamexico@gmail.com