Artemisia Extracts and Artemisinin-Based Antimalarials for COVID-19 Management

Subjects: Others

Contributor: Pawan K. Agrawal, Chandan Agrawal, Gerald Blunden

Artemisia annua (“sweet wormwood”, “qinghao”), a member of the Asteraceae family, has been traditionally used safely over the centuries to treat a variety of fevers, and notably, “intermittent fevers” and chills-related conditions, including respiratory tract infections. It also exhibit positive effects against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and COVID-19 related symptoms. *A. annua* is a source of artemisinin, which is active against malaria, and also exhibits potential to fight against the SARS-CoV-2 infection by inhibiting its invasion, and replication, as well as reducing oxidative stress and inflammation, and mitigating lung damage.

Keywords: Artemisia ; artemisinins ; antiviral

1. Introduction

One of the biggest breakthroughs in fighting the COVID-19 pandemic has been the development of vaccines that provide the best strategies to prevent infection against COVID-19. Several vaccines have been approved by the U.S. Food and Drug Administration (FDA). These have been shown to be highly effective and are available to the public for emergency use authorization (EUA) and for protection against COVID-19 [1]. These vaccines are safe and effective, since, in the rare instances of breakthrough infections (where a person has been vaccinated against COVID-19), patients are significantly less likely to become hospitalized. While vaccines prevent disease occurrence, infected individuals still need other treatment options.

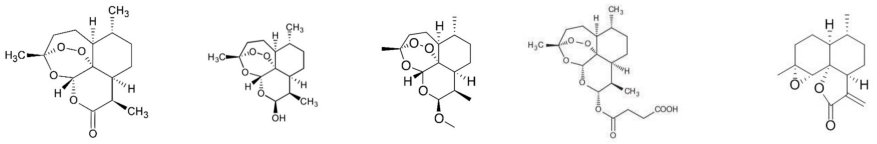
Viral infection happens when a virus inserts its genetic code into a host cell, forcing it to replicate, produce more viral genomic material, and then leads to the death of the host cell. During a viral infection, this process can happen at enormous rates, which leads to viral fever affecting primarily the respiratory tract system, harmful inflammation, and excessive aberrant immunological responses as the body’s immune system tries to seek out and destroy viral material and, at a later stage, it can lead to potentially deadly complications [2][3][4]. By preventing virus entry and/or its replication or clearing of cells into which the virus has already entered, effective treatments with antivirals can help to slow the spread of a person’s infection, potentially reducing the length and severity of symptoms. Thus, safe and effective antivirals responsible for restricting viral entry and/or disruption of the replication process are crucial to the pandemic response [5][6][7][8][9][10][11][12][13].

Despite vaccine developments, COVID-19 treatment still remains largely supportive with an urgent need to identify effective anticoronavirals. An attractive approach is repurposing drugs already licensed for other diseases. In this respect, several studies have been undertaken to test whether antimalarial drugs could treat COVID-19. Teas of *Artemisia annua* L. plants have been employed to treat malaria [14][15][16] in many African countries. Scientists are currently testing *A. annua*’s potential against SARS-CoV-2, as it provides a basis for a large variety of derivatives used as antimalarial drugs, collectively called “artemisinins”.

2. Traditional Use and Bioactive Compounds of *Artemisia*

Artemisia annua (“sweet wormwood”, “qinghao”), a member of the Asteraceae family, has been traditionally used safely over the centuries to treat a variety of fevers, and notably, “intermittent fevers” and chills-related conditions, including respiratory tract infections [17][18][19][20]. One of the most bioactive compounds identified is a sesquiterpenoid lactone, artemisinin (**1**), which contains an unusual 1,2,4-trioxane moiety with an endoperoxide group. This compound has been identified as an active ingredient to treat malaria infections. This unusual endoperoxide bridge is the key active site for its drug mechanism of action and provides a structural chemical base for the synthesizing of a large variety of compounds, such as dihydroartemisinin (**2**), β-artemether (**3**), and artesunate (**4**) (**Figure 1**), exhibiting greater potency, improved water solubility, and improved pharmacological properties [21]. These artemisinin derivatives are the components of

artemisinin-based combination therapies (ACTs), which have been approved as front-line drugs for treating *Plasmodium falciparum* malaria [22][23][24]. They also show additional pharmacological benefits such as anticancer, anti-inflammatory, and immunomodulatory properties [25][26][27][28][29][30][31][32][33]. In addition, *A. annua* has been extensively investigated and more than 600 chemical constituents have been identified [18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40].



(**1**) (**2**) (**3**) (**4**) (**5**)

Figure 1. Chemical structure of artemisinin (**1**), dihydroartemisinin (**2**), artemether (**3**), artesunate (**4**), and arteannuanin B (**5**).

The artemisinin derivatives artesunate and artemether are the key ingredients of the WHO-recommended antimalaria combination therapies [41][42]. *A. annua* extracts and their constituents are active against several viruses, including SARS-CoV [43][44][45], suggesting the usefulness of artemisinin's potential for drug repurposing.

3. Anti-Viral and Immune-Stimulatory Potential of *Artemisia*

In 2002, Lin et al., reported the use of *A. annua* against SARS coronavirus [46]. Interestingly, in a Vero cell-based, 3-(4,5-dimethylthiazol-2-yl-)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) assay for virus-induced cytopathic effect (CPE) screening analysis of medicinal plant extracts with antiviral potentials against SAR-CoV viral strain BJ001, *A. annua*, alongside three other plants, demonstrated a substantial inhibitory effect [46]. The ethanolic extract of whole plants of *A. annua* showed potent antiviral activities with 50% effective concentration (EC_{50}) values of 34.5 (±2.6) and 39.2 (±4.1) µg/mL against the SARS-CoV-1 viral strains BJ-001 and BJ-006, respectively, with a CC_{50} value of 1053.0 ± 92.8 µg/mL in a cytotoxicity assay [47]. Ethnopharmacological studies of *Artemisia* and its constituents have also revolved around their retroviral properties [43][47][48][49][50], capacity to minimize the replication of herpes viruses [43][51][52][53][54], and activity against bovine viral diarrhoea, Epstein–Barr virus, hepatitis B virus, and hepatitis C virus [55][56][57][58][59][60][61][62]. Interestingly, derivatization enhanced the antiviral activity of artemisinin as its derivatives, i.e., artesunate, artemether, and arteether, including dimer and trimer molecules, exhibited potent antiviral activities [62]. For example, artesunate effectively inhibits human cytomegalovirus (HCMV), human herpes simplex virus (HSV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and polyomavirus BK [43][63][64]. Dihydroartemisinin has also shown inhibitory effects on HCMV and Zika virus [65][66], whereas artemisone, alone and in combination with other anti-HCMV agents, has been proven to be a potent HCMV inhibitor [67][68]. Artemisinin inhibited the replication of hepatitis C replicon, a single-stranded RNA virus, similar to SARS-CoV-2 [69]. A recent review by Efferth provided up-to-date information about the inhibition of viruses by artemisinin-type compounds [62].

The presence of flavonoids, such as quercetin and rutin, in *Artemisia* species can be associated with inhibition of activity of the main protease (M^{pro}), also known as chymotrypsin-like protease (CL^{pro}), an enzyme intrinsic for replication of SARS-CoV-2 [70][71][72][73][74]. The presence of various bioactive components in *A. annua* seems to be responsible for its adoption as a therapeutic option against coronavirus infection. *Artemisia* also contains a high concentration of zinc, which has been reported to have an immunomodulation effect on the host response [75]. It should also be noted that the antioxidant ability of *Artemisia* has been shown to enhance immune defence [30][76]. The tea infusion of *A. annua* has shown potent anti-HIV activity, with a half maximal inhibitory concentration (IC_{50}) of 2.0–14.8 µg/mL in vitro. The tea infusion was lacking in artemisinin, suggesting that the anti-HIV activity may be associated with other compounds [50].

Thus, the antiviral and antimalarial significance of *A. annua* and artemisinin derivatives have led to exploring their diverse pharmaceutical potentials [76][77]. Furthermore, earlier pharmacokinetic, pharmacodynamic, and cytotoxicity studies have identified additional factors that made them potential candidates for drug repurposing [78][79][80][81]. Thus, the COVID-19 pandemic outbreak has attracted attention on the efficacy and repurposing of the multifunctional properties of *Artemisia* and artemisinin-derived products as promising therapeutic drugs for the possible treatment of SARS-COV-2 [81][82].

Some antimalarial and/or antiviral agents, such as chloroquine (CQ), hydroxychloroquine (HCQ), and redmesivir, have been repurposed for their possible use against COVID-19 [83]. However, these may have caused cardiotoxicity concerns,

as well as other after-administration side effects [84]. However, notably, artemisinin has been reported to possess a better and lower toxicity profile [85].

4. *Artemisia* Extracts and COVID-19

In response to the pandemic, in April 2020, a herbal tea or decoction based on *Artemisia*, developed by the Malagasy Institute for Applied Research (IMRA), and branded as “COVID-Organics”, was launched as a cure for COVID-19. It contains 62% *Artemisia annua* and a mixture, in confidential proportions, of Malagasy medicinal plants used in the composition of traditional remedies, such as antiseptics and bronchial fluidizers. President Rajoelina of Madagascar said that trials conducted on the COVID-Organics drink showed its effectiveness against the disease [86]. However, the use of a tonic containing unknown quantities of artemisinin and other constituents, over a large population, certainly raises fears of malarial parasites developing resistance. Moreover, its widespread unregulated usage as remedies for malaria, such as in tea, could result in reduced access to effective medicines and possible resistance of *P. falciparum* to artemisinin-based combination therapies (ACTs) [87][88][89]. Since May 2020, IMRA has been preparing an injectable form of *Artemisia*-derived products for patients in respiratory distress. In a recent study by Nie et al., it was shown that several *Artemisia* extracts, as well as Covid-Organics, at concentrations that did not affect cell viability, inhibited SARS-CoV-2 and feline coronavirus (FCoV) infection [90].

In a study related to the efficacy of *A. annua* extracts in high-throughput antiviral in vitro assays in VeroE6 cells, Gilmore et al., found that the leaves, after being extracted with either pure ethanol or distilled water, showed antiviral activity and the activity increased considerably when the ethanol extract was combined with coffee [91]. Extracts were added to VeroE6 cells either 1.5 h prior to infection (pretreatment (pt)] or 1 h post infection (treatment (t)), followed by a two-day incubation of the virus with extracts. The EC_{50} values were 173 µg/mL (pt) and 142 µg/mL (t) for the ethanolic extract; 390 µg/mL (pt) and 260 µg/mL (t) for the aqueous extract; and 176 µg/mL (pt) and 128 µg/mL (t) for the ethanolic extract and coffee, respectively [91]. With all extracts, almost complete virus inhibition was achieved at high concentrations: Cell viability assays revealed median cytotoxic concentrations (CC_{50}) of 1044 µg/mL (*A. annua* ethanolic extract), 632 µg/mL (*A. annua* + coffee ethanolic extract), and 2721 µg/mL (*A. annua* aqueous extract). Selectivity indexes (SI), determined by dividing CC_{50} by EC_{50}, revealed similar results. For the *A. annua* ethanolic extract the SIs were 6 (pt) and 7 (t), for the *A. annua* + coffee ethanolic extract 3 (pt) and 5 (t), and for the *A. annua* aqueous extract 7 (pt) and 10 (t), respectively [92]. The use of dried *A. annua* leaves has also been suggested as a potential therapeutic and inexpensive option for treating SARS-CoV-2 infection [92].

Recently, hot water extract obtained from dried leaves of *A. annua*, obtained from four different parts of the world, was tested against SARS-CoV-2, and two variants, B1.1.7 and B1.351, showed IC_{50} values corresponding to <12 µM artemisinin [93][94][95]. It was also noticed that the antiviral effect of the extracts decreased in inverse correlation with the artemisinin content. The failure of the IC_{50} to decrease as the concentration of artemisinin and/or flavonoids increased, indicated that these were not the only active factors, but may, in fact, be antagonists of the bioactive component. The plant possesses compounds that inhibit inflammation and the formation of scar-like tissues known as fibrosis, which also affect patients with COVID-19, but this warrants further investigation [93][94][95].

In South Africa, teas of *Artemisia afra* were used without in vitro or clinical data [96]. *A. afra*, in contrast to *A. annua*, does not contain artemisinin. Due to fears that artemisinin combination therapies against malaria may become ineffective if artemisinin-based treatments are used against COVID-19 [97], the WHO recently called for investigations into the efficacy of plant-based traditional medicines [98]. Human clinical trials will be required to answer the question whether the traditional medicines may indeed have an effect in either preventing or treating COVID-19 infections.

A study by Zhou et al. [99] related to the in vitro efficacy of *A. annua* ethanolic and aqueous extracts, artemisinin, artesunate, and artemether against SARS-CoV-2 spike glycoprotein revealed that treatment with extracts and compounds inhibited SARS-CoV-2 infection of VeroE6 cells, human hepatoma Huh7.5 cells, and human lung cancer A549-hACE2 cells. In treatment assays, the range of 50% effective concentrations (EC_{50}) ranged between 83 and 260 µg/mL for *A. annua* extracts [99].

The aqueous fraction of *A. annua*, after the extraction of artemisinin, has been shown to regulate the expression of proinflammatory cytokines, matrix metalloproteinases, and NF-κB; to promote cell cycle arrest; to drive reactive oxygen species production; and to induce Bak or Bax-apoptosis [17]. It has also been reported that among the three different ethanol extracts (50%, 70%, and 95%), only the 70% and 95% extracts showed any positive antiviral activity, and the 70% extract was considered to be optimum for further investigation, as the 95% ethanol extract could be associated with cellular toxicity [100].

In a recent study, hot-water extracts of *A. annua* were found to be active against SARS-CoV-2 and its alpha, beta, gamma, delta, and kappa variants. The *A. annua* cultivar with the lowest artemisinin content had the lowest (most effective) IC_{50} against gamma, delta, and kappa variants, thus, demonstrating the potential of the extracts as treatments against this virus [101]. However, clinical studies are required to further evaluate the utility of these teas/drinks/extracts for COVID-19 prevention.

5. *Artemisia* Supplement and Formulation

The Max Planck Institute of Colloids and Interfaces (Germany) is collaborating with a company in the USA, ArtemiLife Inc., to explore the effect of *A. annua* plant extract and artemisinin derivatives against SARS-CoV-2 [102]. ArtemiLife is also marketing *A. annua* herbal tea and coffee directly to consumers, but is careful to note that its tea and coffee are "not intended to diagnose, treat, cure or prevent any disease" and cautions that common side effects may include hearing loss and liver problems. However, it also claims that drinking two servings per day will help consumers "maintain an active shield," thus, "protecting well-being." The firm's coffee contains 0.4 g *A. annua* per serving, and its tea containes 0.23 g. The dried leaves of *A. annua* usually contain around 1% artemisinin, therefore, consuming the drinks would offer much lower doses than typical ACTs [103].

The product, ARTIVeda/PulmoHeal, delivered in a gelatin capsule, is an Ayurvedic drug against COVID-19. The drug is a formulated extract of *Artemisia* for oral delivery of artemisinin for growth factor-β (TGF-β) inhibition. It targets COVID-19 by suppressing both viral replication and clinical symptoms, i.e., both viral and immune driven pathologies (ARDS and cytokine storm) that arise from viral infection. With treatment, viral replication is suppressed, IFNβ is induced, and innate and adaptive immune responses are suppressed. The clinical studies on patients with mild and moderate COVID-19 have suggested that administration of artemisinin 500 mg capsules once daily for 5 days may lead to a faster recovery [103].

In a controlled Phase II trial, patients with COVID-19 received ArtemiC, a medical spray (containing artemisinin, curcumin, frankincense resin from the *Boswellia sacra* tree, and vitamin C, in a nanoparticular formulation for spray administration), in addition to standard care; improvement in the patients' condition was recorded [104].

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