https://doi.org/10.31925/farmacia.2022.3.3 **REVIEW**

ARTEMISIA SPECIES AND ARTEMISININ DERIVATIVES AS ANTIVIRAL AGENTS AGAINST COVID-19. CURRENT KNOWLEDGE: CHEMISTRY, PHARMACOLOGY AND CLINICAL EVIDENCE

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Manuscript received: November 2021

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been the reason behind the third zoonotic outbreak from the *Coronaviridae* family during the 21st century. COVID-19 was declared a pandemic by the WHO on 11 March 2020, and it has been posing a challenge for the health care system all around the world. Research groups worldwide are trying to find potential leads for drugs against SARS-CoV-2. Investigating natural products and extracts from known medicinal plants has been one area of focus in searching for potential inhibitors of SARS-CoV-2. This review aims to highlight the importance of including natural plants and plants derived compounds in this investigation. We particularly focused on compounds from *Artemisia* species (artemisinin and its derivatives), as this plant is highly valuable and has various pharmacological profiles.

Rezumat

SARS-CoV-2 (*Coronaviridae*) este agentul etiologic al pamdemiei COVID-19, o provocare pentru sistemul de sănătate din întreaga lume. Investigarea produselor naturale și a extractelor din plante medicinale cunoscute este un domeniu de interes în iderntificarea potențialilor inhibitori SARS-CoV-2. Acest *review* analizează compușii din speciile *Artemisia* (artemisinina și derivații săi), datorită diversității profilurilor fitochimic și farmacologic.

Keywords: COVID-19, SARS-CoV-2, *Artemisia*, artemisinin, anti-viral

Introduction

Declared a pandemic by the WHO on 11 March 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused 452,201,564 cases world-wide and about 6,029,852 deaths up to date [1]. Being the cause of the 3rd outbreak caused by the *Coronaviridae* family during the 21st century, SARS-CoV-2 has a basic reproductive rate (R0) between 2 and 4 depending on the variant, making it harder to contain, as it is highly contagious [2]. SARS-CoV-2 is a positive-sense single-stranded RNA and a member of the Beta corona-virus genus. It is closely comparable to the two other viruses responsible for the previous coronavirus out-breaks: acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome coronavirus (MERS-CoV). There is a 79.5% similarity with SARS-CoV with a 94.6% resemblance in the amino acid sequence of seven conserved non-structural proteins [3]. This information has been quite helpful during the search for potential inhibitors of SARS-CoV-2.

COVID-19 has affected all aspects of our lives, with a deleterious effect on society, economy and geopolitics. This may also be attributed to the precaution measures put in place by the CDC and WHO, such as the lock-down and quarantine. While this measure remained crucial for the longest time for the safety of the community as a whole and was a cornerstone for combating the virus, they are also expected to cause severe psychological effects on the population, including, but not limited to acute panic, obsessive behaviours, depression and post-traumatic stress disorder (PTSD) among the population [4].

The most frequently reported symptoms of COVID-19 are fever, cough of a dry nature and fatigue. More severe symptoms most prominently include shortness of breath, which is mainly present in the older population, where about 80% of hospitalized cases are of individuals above the age of 65. This age group has a 23-fold increased risk of being infected and manifesting the critical symptoms mentioned [5]. However, the elderly are not the only vulnerable group to SARS-CoV-2, but patients with existing health conditions and co-morbidities are also highly vulnerable. This includes any chronic condition that undermines the body's immunity, such as diabetes, obesity and cardiovascular diseases [6].

For the longest time of the pandemic, the focus of the employed treatment worldwide was to alleviate the symptoms of the patient and employ efforts to prevent

the occurrence of coinfection with bacteria. This includes providing breathing support *via* mechanical ventilation and reducing lung swelling by using corticosteroids, and lastly, the use of available antiviral and antibiotics medications [7]. With researchers globally answering the quest to find effective inhibitors against SARS-CoV-2, several agents are now approved by the WHO and are currently approved for use in non-hospitalized patients older than 12 years [8]. Still, the quest of finding more agents is ongoing, trying to investigate as many biologically active compounds and drug sources as possible.

Natural sources, mainly plants, act as a very important source of medicine due to their production of numerous secondary metabolites [9]. In fact, the WHO states that about 80% of the world relies on herbal medicine sources for treatment [10]. Being a centuries-old practice, the use of plants as a medicinal source has led to the discovery many important drugs. Accordingly, even though their mechanism of action may not be fully understood, requiring proper evaluation before potential use, the inclusion of naturally derived products in the search for an anti-SARS-CoV-2 agent should be pursued [11].

The literature is filled with evidence of the effectiveness of natural compounds derived from plants that are effective against respiratory conditions and, more specifically, viral respiratory infections. Research groups worldwide are investigating the efficacy of traditional Chinese medicine (TCM) against COVID-19, as it has shown promising results with SARS-CoV, resulting in shorter hospitalization periods and reduced steroid-related side effects and viral symptoms improvement (World Health Organization, 2004). For instance, one group is studying the potential effects of extracts from the dried fruit of *Forsythiae fructus* [12*]*. *Artemisia vulgaris* is among some of the plants found in the literature that were reported as effective against respiratory conditions [13]. Other examples include, but are not limited to *Boerhavia procumbens* [14], *Capparis spinosa* L. [15] and *Carum copticum* [16]. This knowledge is important as it means that careful evaluation of natural plants and compounds can help discover agents that effectively alleviate the respiratory symptoms of SARS-CoV-2.

Our aim with this review is to highlight the significant potential of *Artemisia* species and other *Artemisia* derivatives as antiviral agents against SARS-CoV-2. As we can see above, they have been identified in the literature as both effective against respiratory conditions and as antiviral agents, meaning that they have the potential to both alleviate the troublesome symptoms suffered by the patient and eradicate the virus from the body, making it an interesting target worth investigating. For instance, Shah *et al*. reported an anti-asthmatic effect attributed to the aerial parts of the *Artemisia maritima*, mediated by bronchodilation [17]. Blockade of calcium channels and phosphodiesterase were the two proposed mechanisms thought to mediate bronchodilation. Additionally, *Artemisia annua* has previously been tested against SARS-CoV and has shown a notable antiviral effect against it [18]. The results were promising, and since there is a 79.5% between both viruses, it is expected that such promising results will be replicated with SARS-CoV-2. Wang *et al*. [19], performed virtual screening of various natural compounds based on the TCM (Traditional Chinese Medicine) database, which identified aurantiamide acetate derived from *A. annua* plant as a SARS-CoV inhibitor, mainly targeting the SARS cathepsin L.

Finally, this plant has been studied extensively against flu-like symptoms, and it has shown significant efficacy against such conditions. Another study reported that *Artemisia*'s effectiveness in antitracheitis (inflammation at the trachea), cough, phlegm removal and asthma relief. Another promising point is that *A. annua* showed a significant *in vitro* effect against SARS-CoV-1 in 2002 with an IC_{50} value of 34.5 ± 2.6 μg/mL) [20].

Methodology

Keywords like *Artemisia*, artemisinin and derivatives, were used in scientific databases like Scopus and PubMed for the initial search for articles about artemisinin and derivatives in general, resulting in about 16,635 articles. The articles found were then filtered using keywords like biological activity and medicinal use, cutting down the number of articles to 5,523. The inclusion criteria for reviewing an article to in include it in the final section of this paper (*Effectiveness of Artemisia Species and Derivatives against COVID-19*), the resulting articles were then further limited by including keywords such as SARS-CoV-2 and COVID-19, which resulted in 82 articles. We tried to include original research articles (excluding book reviews and chapters), published in or after 2020, for the inclusion in the last section, resulting in around 30 articles, that were carefully evaluated to ensure that they fit the scope of our review. The structures of artemisinin and its derivatives were also drawn in SciFinder to aid us in finding articles related to these compounds.

Artemisia as a Medicinal Plant

Artemisia is a member of the *Asteraceae* family consisting of 500+ species [21]. Some of the most notable *Artemisia* species include, but are not limited to *A. absinthium*, *A. abrotanum*, *A. afra*, *A. annua* and *A. vulgaris* [22]. *Artemisia annua* L. is currently among the top 10 pharmaceutical crops, receiving significant attention from researchers worldwide. This is attributed to the fact that it is currently the only source for pharmaceutical production of artemisinin [23]. *Artemisia* is a genus of small herbs and shrubs that grows wild in Asia (mainly China, Japan and

Korea) and was introduced to Poland, Brazil, Spain, France, Italy, Romania, the United States and Austria, where it became domesticated [24]. *Artemisia annua* L. is rich in artemisinin, a member of the *Artemisia* family used in traditional Chinese medicine for thousands of years [25].

Traditional use

Artemisia has appeared in many ancient Chinese medical manuscripts, and some of its traditional uses in traditional Chinese medicine included the treatment of fevers and haemorrhoids [23]. Other manuscripts also describe its benefits in treating wounds, enhancing the brightness of eyes and even improving longevity [26]. Its use was not only limited to that, but it was also an ancient Chinese herbal remedy for pyrexia [27]. Other ancient medical textbooks recommended the use of *A. annua* for bone steaming and heat/fever arising from exhaustion, tuberculosis, lice, wounds, scabies, dysentery, acute convulsions related to pollution through contact with the dead, pain and swelling around the tooth, pus in-ear, rhinopolyp, summer-heat relieving, haemostasis and analgesic some activities [28].

Treatment of different conditions

Artemisia has diverse biological effects, from anti-cancer to antimalarial activities [29]. Their leaves are rich in flavonoids with excellent antioxidant activity [30].

Many if not all of the pharmacological effects of *Artemisia* are brought on by its secondary metabolites. Some phenolics from *A. annua* consist of coumarins, flavones, flavonols, phenolic acids and miscellaneous. The *Artemisia* pharmacological properties can be summarized as follows; anti-bacterial, anti-inflammatory, antitumour, anti-protozoa, anti-helminthic, anti-fungal, anti-angiogenic and antiproliferation properties. Additionally, the effectiveness of artemisinin and its derivatives against obesity and metabolic disorders has gained a lot of interest in recent years.

Liver cancer, brain glioma, leukaemia, nasopharyngeal cancer, gallbladder cancer, gastric cancer, cervical cancer, lung cancer, breast cancer and colon cancer are a few examples of cancers that artemisinin can be used against [23].

Artemisinin family drugs have shown effectiveness in treating autoimmune diseases [31]. Wu *et al*., described the novel artemisinin derivatives in treating autoimmune diseases [32]. One study reported that artemisinin (ART) and its derivatives are potentially effective drugs for treating helminthic diseases [33]. Keiser *et al*., have reported that ART derivatives and synthetic peroxides such as ozonides and trioxolanes may be used as alternative or complementary drugs against schistosomes (snail fever) [34]. Artemisinin dimmers were also reported to be promising leads for developing effective anticancer agents [35]. Being involved in the iron delivery mechanism occurring intracellularly, artemisinin dimers and trimers and artemisinin hybrid compounds are all promising potent anticancer compounds. Not only that, but they also hold the promise of causing fewer adverse effects compared to traditional chemotherapeutic agents [36]. Many studies have also reported on the effectiveness of artemisinins against CNS related conditions. For instance, some studies reported the efficacy of artemisinins in treating neuro inflammation-related central nerve system (CNS) diseases [37]. Another study reports strong evidence for the potential use of artemisinin B in treating neuroinflammatory diseases [38].

Finally, one study also reported the liver protective effect of artemisinin, noting the enhanced stability and reduced damage to the liver cell membrane and cells. It also reported the protective effect of artemisinin against chronic alcohol poisoning and the clinical potential of treating liver injuries induced by alcohol [39].

Chemistry of *Artemisia*

Chemical classification

Artemisinin, isolated from *A. annua* leaves (Qinghaosu), is a sesquiterpene lactone compound characterised by an endoperoxide bridge [40]. Due to the C–O–O–C complex formula of artemisinin, the chemical synthesis is complicated with a low percent yield [41]. Artemisinin contains a 15C atom formed from combined isoprene units (5C building blocks). Artemisinin has a tetra-cyclic structure characterised by one trioxane ring and one lactone ring. The endoperoxide bridge found in the trioxane ring is the active moiety of the artemisinin [42].

The complete biosynthetic pathway of artemisinin is generally divided into three main steps, (1) the production of farnesyl pyrophosphate (FPP) *via* mevalonate and non-mevalonate pathways (DXB path-way), (2) the cyclisation of FPP to amorphadiene, (3) the conversion of amorphadiene into artemisinin acid and its derivatives, dihydroartemisinic acid and artemisinin, as shown in Figure 1 [43]. The exact biosynthesis of artemisinin is not fully clear. However, the main steps provided by the study of Nishi Srivastava [43] are the steps confirmed so far by the researchers. The biosynthetic pathway starts with the Linear isoprene precursors, including FPP, Geranylgeranyl diphosphate (GPP) and geranylgeranyl diphosphate (GGPP), that are produced by both the mevalonic and the plastidic, non-mevalonic pathway, also known as methylerythritol phosphate (MEP) or deoxyxylulose phosphate (DXB) pathway. The isopentenyl diphosphate (IPP) precursor of mevalonate origin produces FPP, while the non-mevalonic pathway gives rise to the precursors of GPP and GGPP [44]. Recent evidence proved the crosstalk between the two pathways to provide the FPP compound [45].

Briefly, IPP and dimethylallyl diphosphate (DMAPP) form FPP, the key precursor for artemisinin biosynthesis. FPP is then converted to amorpha-4,11-diene through amorphadiene synthase (ADS). ADS enzyme is crucial for artemisinin synthesis. It is the first limiting step of this process [46]. Interestingly, ADS is found to be accumulated in leaves by 16-fold and 10-fold higher than in roots and stems, respectively, which produce what we call a tissue-specific expression pattern [47]. After forming amorphadiene, several steps of oxidation and hydroxylation *via* CYP450 enzymes, such as CYP71AV1, occur to produce both artemisinic alcohol and artemisinic aldehyde [46]. The latter two compounds are reduced to give arteanniun B, artemisitene and artemisinin, as shown in Figure 1.

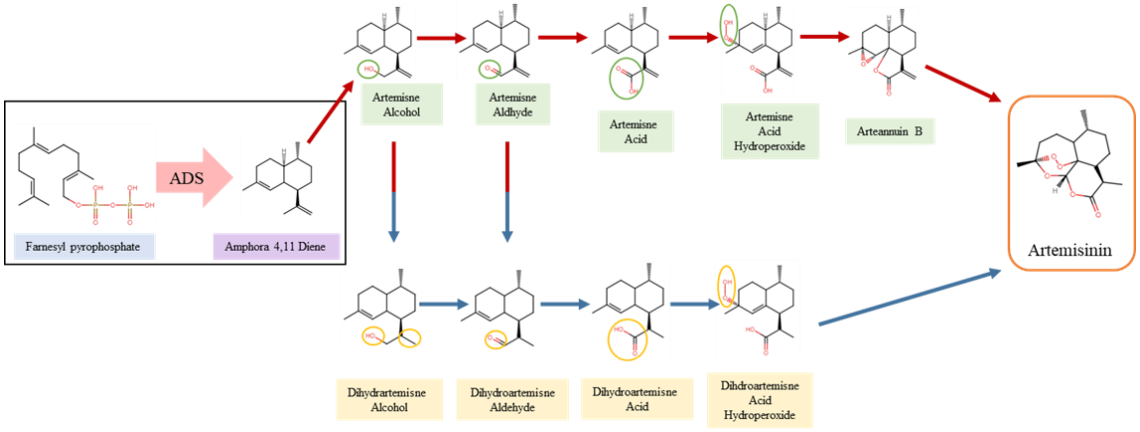

Figure 1.
The conversion of FPP to amorphadiene *via* ADS, and the biosynthesis of artemisinin from amorphadiene

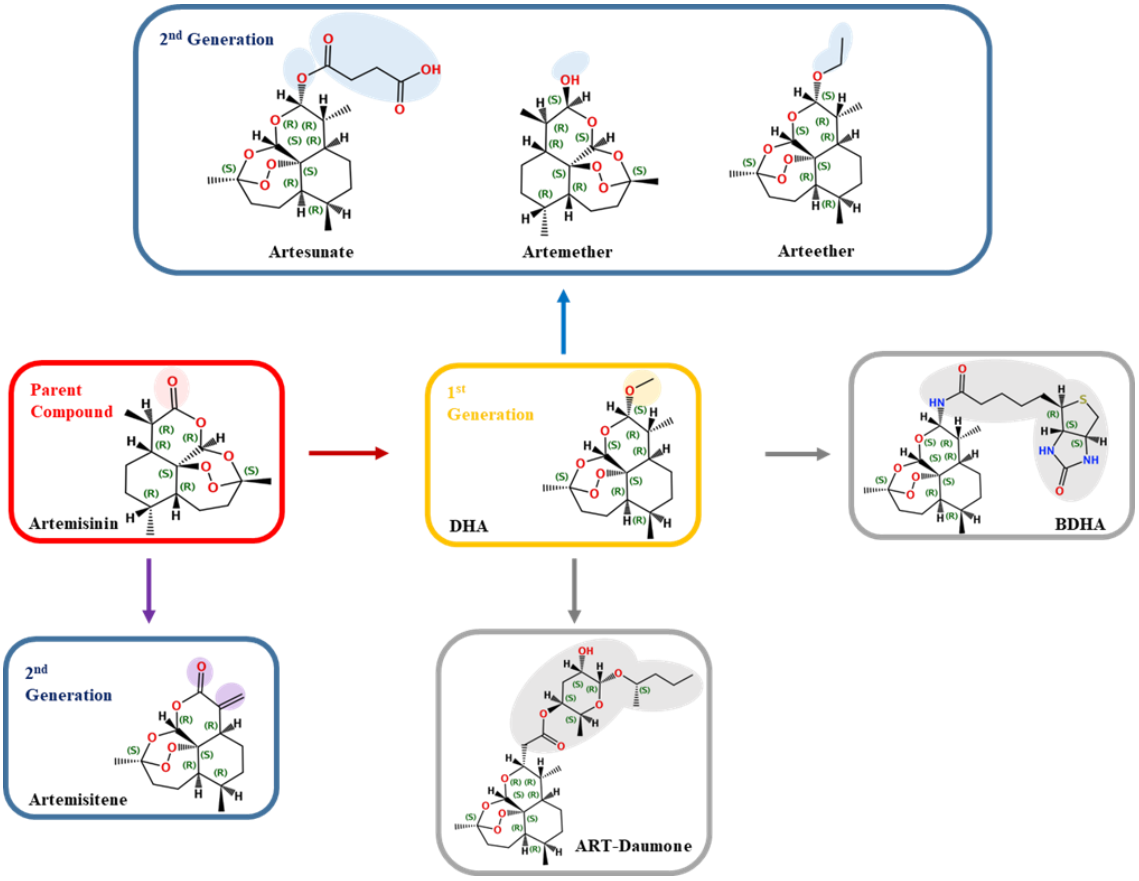

Figure 2.
Chemical structures of artemisinin and its derivatives

Chemical structures of artemisinin and its derivatives

The first isolated compound from *A. annua* was artemisinin, the parent compound in this class [48]. Artemisinin has poor absorption due to low water/oil solubility and short half-life [49]. To overcome this problem, chemical modification of the structure

was needed. The produced derivatives should maintain the peroxide bridge, which is vital for the compound's activity [50]. The first generation, dihydroartemisinin (DHA), was produced by modifying the carbonyl group of artemisinin into a hydroxyl group or reducing the lactone ring to a lactol ring as shown in Figure 2 [51]. The second generation of derivatives is more potent than artemisinin and easier to be synthesized [52]. These derivatives have developed properties like better water solubility (artesunate), higher lipid solubility (artemether and arteether) and better pharmacokinetic-pharmacodynamic profiles [52].

Pharmacokinetic & Pharmacodynamic Profiles of Artemisinin and Its Derivatives

Artemisinin is not completely absorbed after oral ingestion [53]. It can be taken rectally and IM but no intravenous dosage form is available. It is known to have a passive diffusion through cell membranes [54]. Artemisinin crosses both placental and blood-brain barriers [55]. Artemisinin is metabolized by CYP2B6, CYP3A4 and CYP2A6. In addition, has a rapid elimination. Unlike other derivatives, DHA has only one route of administration, the oral route. DHA is metabolized to artemether and artesunate. However, DHA has longer half-life compared to artemether and artesunate [56]. Artesunate, a prodrug for DHA, can be taken orally, rectally, intramuscularly and intravenously [57]. Artesunate is a succinate water-soluble compound and has a faster activity than artemisinin and artemether. Artesunate effective anti-malarial regimen is 10 mg/kg for 5 to 7 days [58]. Combination therapy with mefloquine, for a minimum of 3 days, is preferred for multi-resistant strains of parasites [59]. Conversely, artemether and arteether are oil-soluble derivatives. Oral artemether is incompletely absorbed and rapidly eliminated. Artemether is a methyl ether derivative of DHA, it forms DHA that is found to have higher plasma concentration than the given artemether [53]. Arteether, also known as artemotil, is the oil-soluble ethyl ether derivative of DHA. Arteether has a favourable slower elimination rate and less toxicity because it is metabolized to ethanol not methanol of artemether. Another advantage of arteether over artemether is the higher lipophilicity which increases the drug accumulation in body tissues [60].

General Pharmacology

The first therapeutic use of artemisinin was as an anti-malarial agent. Qinghaosu was discovered in China by You-You Tu in the early seventies. The anti-malarial mechanism of action is generally related to the formation of free radicals by the cleavage of the Endoperoxide Bridge of the Trioxane Bridge, which is catalysed by parasitic heme-ion. The free radicals then alkylate and disturb some essential parasitic proteins leading to alteration in the normal biochemical process of the parasite, thus induce its death [61].

Pathway alteration mechanisms are still not absolutely confirmed. Studies listed some mechanisms including alteration in parasite sarcoplasmic or endoplasmic calcium ATPase, changes in the mitochondrial respiratory chain, production of reactive oxygen groups and effects on immunity and angiogenesis [62]. These mechanisms were found to affect other pathological cells as tumour cells. Due to that, further studies of the non-malarial activities of Qinghaosu were conducted.

Anti-inflammatory effects of artemisinins have been tested due to their safe profiles and potent activity on various signalling pathways [45]. Artesunate possess immunosuppression effect by inhibiting certain spleen cells and lymphocytes. Subsequently, other derivatives were found to have the same activity. Artemisinin and derivatives can help in rheumatoid arthritis through reducing the inflammatory symptoms, tissue oedemas and cartilage destruction. This occurs by the suppression of some proinflammatory cytokines including TNF-α, IL-1β, GM-CSF and IL-6, and inhibition of NF-κB signalling pathways [63, 64]. Fortunately, the side effects caused by the standard therapy of rheumatoid arthritis, methotrexate, were found to be minimized with the use of artemisinin therapy [65]. Artesunate was discovered to be effective against allergic contact dermatitis [66]. Further study discovered the effectiveness of artesunate against other skin conditions like eczema, polymorphous sunlight eruption and atopic dermatitis [67]. A study in 2013 focused on the use of artemisinin in Alzheimer's disease. Artemisinin diminished the levels of neuroinflammation and amyloidgenesis remarkably [68]. The activity of artemisinins exceeded expectations by having a prominent effect on parasitic diseases, such as schistosomiasis, that are phylogenetically unlinked to malaria. Artemisinins were found to block the growth of two trypanosome species *T. brucei* and *T. cruzi*, the cause of the African trypanosomiases or sleeping sickness [69]. The anti-parasitic effect is associated to the inhibition of the Ca-dependent ATPase in parasite membrane, also the mitochondrial damage similarly to the anti-malarial mechanism. *Toxoplasma gondii* is a reason for chronic asymptomatic infections. *T. gondii* is sensitive to artemisinins. Out of the derivatives, artemether is 100-folds more potent than the first-line treatment trimethoprim [70]. Also, artemisinins are effective against both Schistosomiasis infection and the food-borne Fasciola infection [71].

Moreover, artemisinins have cytotoxic effect against various cancer types, such as breast, colon, leukaemia, ovarian cancers and many others. The believed mechanism of action is *via* the formation of ROS caused by the Endoperoxide Bridge. The hydrophobicity of artemisinin enhanced their penetration into cellular membranes, allowing them to have a better anticancer function [72]. Further mechanisms are related to

decrease in cell proliferation, inhibition of angiogenesis and apoptosis induction in cancerous cells [73, 74]. Artemisinin targeted delivery *via* transferrin conjugation was proved to have better anti-cancer results than artemisinin alone [75]. Using transferrin conjugation, artemisinin function as a prodrug that is selectively transported by TfR-mediated endocytosis to cancer cells [76]. A study by Roxana Stan examined the anti-tumour effect of the *Artemisia annua* L. extract. Their results indicated that *Artemisia* enhances the anti-proliferative effect of doxorubicin; therefore the combination therapy is advised [77].

Lastly, *Artemisia*, especialy *artesunate*, have strong inhibition effects on the replication process of Herpes viruses like cytomegalovirus (CMV), human herpesvirus 6A (HHC-6a), and herpes simplex virus 1 (HSV-1). Further detected antiviral activities are on Hepatitis C and B viruses [78], and bovine viral diarrhoea [79]. The recent coronavirus pandemic 2019 has an impact on many lives globally. Since SARS-CoV-2 is a novel virus, thus there is a high need for urgent treatment. Drugs used for malaria, inflammation and antivirals have been tested against coronavirus. Among other anti-malarial agents, artemisinin is distinguished for having less side effects, this allowing the prescription of higher doses without the need to worry about the side effects. Artemisinin treats fevers and respiratory conditions, which are the main symptoms of Corona infections. The anti-viral activity is mediated through reduction in TNF-α and IL-6, the lead causes of acute respiratory distress syndrome [80]. COVID-19 infection increases the formation of oxygen free radicals and increases inflammation, producing a negative impact on the progression of diabetes mellitus and associated consequences [81].

Toxicity and adverse effects

Generally, artemisinin and its derivatives are safer than other anti-malarial drugs like chloroquine. Toxicological studies on rats showed signs of cardio-toxicity and neurological syndrome on acute lethal doses. A similar study on dogs demonstrated neuro-pathological damages in their brains that is considered to be caused by the endoperoxide bridge [82]. Another study on monkeys revealed susceptible haemolysis on high doses of artemisinin and derivatives, except artemether which showed lower haemolysis rate [83]. However, clinical studies on humans reported minimal side effects compared to animal studies. A study on children reported convulsions with treatment using artemether [84]. Cardiotoxicity in humans is limited to prolongation in QT interval, especially with artemether. Still, cardio-effects are not clinically important and only cautions are needed for combination with other drugs with the same cardiotoxicity effects [85]. Despite all the aforementioned conditions, no significant adverse effects have been confirmed in humans in therapeutic doses.

Effectiveness of *Artemisia* Species and Derivatives against SARS-CoV-2

The significance of *Artemisia* species and artemisinin derivatives in treating COVID-19 has been well reported in many studies. A number of research studies revealed how *Artemisia* species and artemisinin derivatives could play a significant role in reducing coronavirus prevalence.

Clinical studies

A recently published study conducted a clinical trial, including forty-one participants infected with corona-virus. Further, the participants were divided into two groups; eighteen subjects received placebo and were considered as the control group and twenty-three subjects received a combination therapy of artemisinin and piperaquine and were considered as the experimental group. On the first day, a loading dose containing two tablets of artemisinin 125 mg and piperaquine 750 mg were administered orally followed by a low dose of one tablet *per* day containing artemisinin 62.5 and piperaquine 375 mg for six days. The study outcomes were based on monitoring the percentage of participants recovered from coronavirus on days seven, ten, fourteen and twenty-eight following the treatment. Finally, the results of the study indicated that the average time to achieve coronavirus recovery in the experimental group was significantly less than in the control group. Additionally, the elimination rate of coronavirus RNA in the experimental group was significantly higher than that in the control group and the length of hospital stay for the experimental group was significantly lower than that in the control group. Although due to the sample size and trial design insufficiency, however, the immune regulatory activity and safe toxicity profile of artemisinin and piperaquine makes them an excellent combination therapy against coronavirus infection [86]. It is noteworthy to mention, that artemisinins are able to block tissue fibrosis, works as an antioxidant, anti-inflammatory and have low toxicity profile for all these mentioned properties, artemisinins are an excellent drug candidate against coronavirus infection [87].

In vitro studies

A research conducted an *in vitro* study to examine whether *A. annua* is able to suppress SARS-CoV-2. The study measured the antiviral activity against fully infectious coronavirus cells by using Vero E6 and Calu-3 cells with dried leaf extracts of seven cultivars of *A. annua* sourced from four continents. IC_{50}s were measured and it revealed the concentrations at which 50% of the viral replication is inhibited. The results of the study indicated that artemisinin and dry leaf mass of hot water leaf extracts showed antiviral activity with IC_{50} values of 0.1 - 8.7 μM. However, the anti-viral activity did not correlate to the extract contents of artemisinin or total flavonoid. Artemisinin alone showed an IC_{50} of 70 μM, and the clinically used

artemisinin derivatives such as artemether, dihydro-artemisinin and artesunate were in effective at high concentrations. This indicates that the antiviral activity in the extracts arises from something besides artemisinin or a combination of components that is responsible for blocking the viral infection before viral entry. In addition, concentrations of artemisinin and total flavonoids varied in the extracts by nearly 100 folds and all hot water extracts were effective. To sum up, further in vivo studies are needed to determine whether *A. annua* is able to provide a cost effective treatment against coronavirus [88].

Another study setting out to investigate *A. annua* extracts as well as artemisinin, artesunate and artemether against SARS-CoV-2, using concentration–response antiviral treatment assays, on VeroE6 cells, human hepatoma Huh7.5 cells and human lung cancer A549-hACE2 cells. Artesunate was found to be the most potent of all tested compounds, with an EC50 ranging from 7 - 12 μg/mL. this was followed by artemether with an EC_{50} of 53 - 98 μg/mL), 83 - 260 μg/mL for *A. annua* extracts 83 - 260 μg/mL and finally 151 - 208 μg/mL for artemisinin [89].

Another study also used Vero E6 cells to investigate the inhibitory action of *A. annua* dried leaves hot extract against different variants of the virus; alpha, beta, gamma, delta and kappa. The extract was found to be active against all variants, with IC_{50} values ranging from 0.3 to 8.4 μM [90].

Another group set out to apply different extraction methods on *A. annua*, and then determine the artemisinin content in these various extracts, to test them for their inhibition effect against SARS-CoV-2. These extracts were reported to have inhibitory effects against Mpro, but not against Spike protein. The artemisinin content in the extracts with inhibitory effect had very low concentrations of artemisinin, which means that the inhibitory effect is not only due to the artemisinin but rather other compounds related to artemisinin, such as artesunate, arteannuin B and Lumefantrine [91].

In situ studies

Another study examined the antiviral activity of artemisinin and its derivatives as combination therapy against clinically isolated coronavirus strains in Vero E6 cells. The study results revealed that mefloquine-artesunate exhibited the highest antiviral activity with inhibition percentage of 72.1 ± 18.3% at drug doses usually administered in normal malaria treatment. In addition, the study revealed that all other artemisinin derivatives combination therapies such as artemether-lumefantrine, dihydroartemisinin-piperaquine, artesunate-pyronaridine or artesunate-amodiaquine exhibited similar antiviral activity with inhibition percentages between 27.1 to 34.1% [92]. In addition, mefloquine-dihydro artemisinin and monodesethyl amodiaquine-dihydro artemisinin were shown to be the best combination therapy as they exhibited significant antiviral activity against coronavirus with inhibition percentage between 99.6% and 85.8%. These *in vitro* results support the hypothesis that artemisinin and its derivatives could be effective drug candidates for coronavirus treatment [92].

Additionally, artesunate is one of the semisynthetic artemisinin derivatives, which is used as an antimalarial drug. Artesunate exerts anti-inflammatory effects by inhibiting interleukin-1beta, interleukin 6, interleukin 8 and tumour necrosis factor alpha as a result of inhibiting nuclear factor kappa B. In addition, it inhibits the mechanism of endocytosis like chloroquine [93, 94]. For all the above-mentioned information, artesunate could be a drug candidate against corona-virus. A study reported that nuclear factor kappa B has been associated in the pathogenesis of many lung diseases, and it has been shown to be affected in both SARS-CoV and MERS-CoV infections [95, 96]. It is noteworthy to mention, that coronavirus mainly involves the upper respiratory tract and causes an elevation in the host immune response. In the host infected with coronavirus, the inflammatory responses usually increases upon the activation of nuclear factor kappa B which significantly affects the progression of the disease [97, 98].

A study supported the significance of artemisinin and its derivatives for their antiviral activity against coronavirus. Artemisinin and seven of its derivatives were examined for their antiviral activity against coronavirus in Vero E6 cells by measuring viral RNA in the supernatant by quantitative real time PCR after 24 hours of treatment. Lumefantrine in combination with artemether was also included in the study. The results revealed that arteanniun B, artesunate and dihydroartemisinin were the most potent derivatives against coronavirus with EC_{50} values of 10 - 14 μm; lumefantrine showed an EC_{50} of 23 μm. In addition, arteanniun B and lumefantrine were found to affect the viral replication of the early entry events. When added post entry they inhibited the viral RNA recovered in the supernatant in addition to viral N protein production visualized by indirect fluorescent antibody and western blot [99].

In silico studies

Finally, a molecular docking study demonstrated how artemisinin and is derived compounds can act as good inhibitors for SARS-CoV-2 spike protein receptor binding domain and compared it with hydroxy-chloroquine. They were found to produce better docking scores compared to hydroxychloroquine. The docking score for artelinic acid was -7.1 kcal/mol and for hydroxychloroquine -5.5 kcal/mol. Artenimol, artesunate and artemisinin exhibited two different ways of interactions, the first with Lys353 and the second with Lys31. These are binding hotspots of the spike protein. Furthermore, molecular dynamics analysis was performed and it confirmed that these formed complexes have the ability to interact and remain

stable in the active site of their targets. Eventually, artemisinin derivatives such as artenimol, artesunate and artemisinin are recommended for the treatment of COVID-19 patients after successful and proved clinical studies due to their antiviral effectiveness and excellent safety profile in humans [100].

Conclusions

In conclusion, this review represents an updated overview about the current knowledge of *Artemisia* species and artemisinins derivatives against coronavirus in term of treatment, pharmacology, chemistry, prevention and clinical evidences. Due to *Artemisia* various therapeutic uses, it is notably used in China and in other herbal medicinal systems. It owes a very promising pharmacological action due to the presence of the lactone derivative in artemisinin and its derived compounds. In this review, we pointed out the significance of *Artemisia* species and artemisinins derivatives towards the treatment of coronavirus proved by many *in vitro* studies and few clinical studies. Hence, further clinical studies are needed. Besides, coronavirus infection is known to be either mild respiratory tract infection with influenza like symptoms or serious life threating viral infection that cause in some cases lung fibrosis, organ failure, neurological problems, inflammatory issues and oxidative stress. Thus, this review proves the significance of *Artemisia* species and artemisinins derived compounds as drug candidates to fight coronavirus infection due to their antioxidant, anti-inflammatory, safety, low toxicity profile and their ability to block tissue fibrosis.

Conflict of interest

The authors declare no conflict of interest.

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