Artemisinins in Combating Viral Infections Like SARS-CoV-2, Inflammation and Cancers and Options to Meet Increased Global Demand

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Artemisinin is a natural bioactive sesquiterpene lactone containing an unusual endoperoxide 1, 2, 4-trioxane ring. It is derived from the herbal medicinal plant Artemisia annua and is best known for its use in treatment of malaria. However, recent studies also indicate the potential for artemisinin and related compounds, commonly referred to as artemisinins, in combating viral infections, inflammation and certain cancers. Moreover, the different potential modes of action of artemisinins make these compounds also potentially relevant to the challenges the world faces in the COVID-19 pandemic. Initial studies indicate positive effects of artemisinin or Artemisia spp. extracts to combat SARS-CoV-2 infection or COVID-19 related symptoms and WHO-supervised clinical studies on the potential of artemisinins to combat COVID-19 are now in progress. However, implementing multiple potential new uses of artemisinins will require effective solutions to boost production, either by enhancing synthesis in A. annua itself or through biotechnological engineering in alternative biosynthesis platforms. Because of this renewed interest in artemisinin and its derivatives, here we review its modes of action, its potential application in different diseases including COVID-19, its biosynthesis and future options to boost production.

Keywords: Artemisia annua, artemisinin, COVID-19, malaria, SARS-CoV-2, sesquiterpene lactone

INTRODUCTION

Artemisinin is an oxygenated sesquiterpene lactone, mostly produced in glandular trichomes (GTs) of the medicinal plant Artemisia annua L. (Tang et al., 2014; Wang et al., 2016; Beyraghdar Kashkooli et al., 2018, 2019). Artemisinin and related compounds derived from the biosynthetic pathway (Figure 1) have been shown to be effective against malaria caused by the Plasmodium spp. parasite (Klayman, 1985; Tu, 2011). The action of artemisinin is not only on the Plasmodium itself, but also because of its effect on human physiology. It is the effects of artemisinin on human physiology that relate to its potential uses in other diseases as well. Below the mode of action of artemisinin and related compounds in Plasmodium and humans are discussed, exemplified by its potential use in the fight against COVID-19.
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GRAPHICAL ABSTRACT | Three anti-SARS-CoV-2 potentials of artemisinin and artesunate. Artemisinin (ART) and artesunate (AS) may (1) block interaction of viral spike protein with the human ACE2 receptors, preventing viral endocytosis and activation of the NF-κB signaling pathway; (2) ART and AS may block activation of NF-κB signaling pathway by IKK, or (3) may interfere directly with p50/p65 transcriptional activity in human cells.

Because of the alternative uses of artemisinin, including the fight against COVID-19, the renewed demand for this compound cannot be met by current production capacity. Therefore, also current production capacity problems and potential solutions are briefly discussed.

ARTEMISININ: MODES OF ACTION IN THE MALARIA Plasmodium PARASITIC CELLS

Artemisinin contains an endoperoxide bridge that is important for anti-malarial activity. In general, several mechanisms of actions have been proposed to explain the bioactivity of artemisinin against Plasmodium spp. (O’Neill et al., 2010). The first proposed action is the interference with Plasmodium mitochondrial and plasma functions (Antoine et al., 2014). Studies have shown that artemisinin/artemisinin semi-synthetic derivatives (totally known as endoperoxides) induce Plasmodium mitochondrial and plasma membrane depolarization (Wang J. et al., 2010; Antoine et al., 2014). These membrane depolarizations are strongly associated with Reactive Oxygen Species (ROS) that are generated by iron bioactivation of the endoperoxides of artemisinin (Antoine et al., 2014; Wang J. et al., 2015; Tilley et al., 2016). An additional mode of action of artemisinin against the onset of malaria is based on the cleavage of the endoperoxide in the artemisinin molecule, resulting in artemisinin free radicals, which act as alkylation agent for susceptible molecules and proteins in the parasitic cell. For instance, the alkylation of Plasmodium falciparum TCTP, ATP6 (a Ca$^{2+}$ transporter) (Shandilya et al., 2013) and PI3K (Mbengue et al., 2015) may interfere with the biological function of these proteins in the infection process. In infected red blood cells, the malaria parasite degrades hemoglobin (as a source of amino acids), resulting in large amounts of free heme molecules. These are potentially toxic to the malaria parasite but are detoxified by the parasite via conversion of heme to hemozoin. The alkylation of heme by activated artemisinin could inhibit this detoxification reaction to hemozoin. In a more general sense, the alkylation of parasitic proteins may also interfere with their correct folding, which in turn may be linked to decreased parasite development. Indeed, treatment with artemisinin results in an upregulation of the Unfolded Protein Response (UPR) (Mok et al., 2015). In addition to artemisinin, dihydroartemisinin attacks parasites by using a two-pronged process, creating protein damage, and endangering parasite proteasome function. The consequent gradual accumulation of proteasome substrates (i.e., polyubiquitinated and unfolded/damaged proteins) results in the endoplasmic reticulum stress and dihydroartemisinin-mediated death of the parasite. Tests with other specific inhibitors of the proteasome create a similar increase of polyubiquitinated proteins, also causing parasite death (Bridgford et al., 2018) (Figure 2).

ARTEMISININS: POTENTIAL MODES OF ACTION IN HUMAN INFLAMMATION RESPONSES

Besides its role in combating malaria, artemisinin has also been investigated for its potential effect on immune responses under physiological and pathological conditions (Effert et al., 2002; Aldieri et al., 2003; Xu et al., 2007; Gu et al., 2012; Lai et al., 2015; Yu et al., 2016; Nunes et al., 2017). Many bacteria and viruses, including the SARS-CoV-2, activate the NF-κB (Nuclear Factor kappa B) signaling pathway in human cells. NF-κB is a transcription factor that regulates multiple aspects of innate and adaptive immune functions and has a central role in inflammatory responses. For instance, NF-κB induces the expression of pro-inflammatory genes like those encoding cytokines and chemokines. NF-κB is a heterodimeric protein complex consisting of p50/p65 which is retained in the cytosol.
FIGURE 1 | Chemical structure of artemisinin and related compounds: artemisinin (A), dihydroartemisinin, another biosynthetic pathway product and also known as dihydroqinghaosu, or artemol (B) and artesunate, which is a semi-synthetic chemical derivative of artemisinin biosynthetic pathway product (C).

FIGURE 2 | Artemisinin mechanism of action against malaria parasite; (i) production of ROS for depolarization of the parasite’s mitochondria, (ii) interference with the heme detoxification pathway of red blood cells (iii) induction of alkylation and inhibition of cellular elements such as PfATPase6 and (iv) via protein damage, and inhibition of parasite proteasome function.

by interaction with IκBα (Verma et al., 1995). Activation of NF-κB signaling activates the IκB kinase activity which results in the release of p50/p65 from IκBα and subsequent movement of p50/p65 to the nucleus where it leads to the expression of specific genes and the production of pro-inflammatory chemokines and cytokines like Interleukin 6 (IL-6) (Verma et al., 1995; Pahl, 1999; Xiong et al., 2010; Liu et al., 2017; Figure 2). IL-6, is a pleiotropic cytokine that is produced in response to infection, tissue-damaging, cellular immune response, and hematopoiesis to contribute and help the host’s defense system
(Tanaka et al., 2014; Velazquez-Salinas et al., 2019). Normally, the production of IL-6 is strictly regulated at transcriptional and post-transcriptional levels. However, certain diseases, like in COVID-19, may cause misregulation of the NF-κB signaling, causing overproduction of IL-6 and other cytokines in a cytokine release syndrome (CRS) (Tanaka et al., 2014; Conti and Younes, 2020; Krishna et al., 2021). Indeed, the dynamic change of IL-6 level can be used as a potential biomarker for a severe case of COVID-19 (Liu et al., 2020; Ulhaq and Soraya, 2020; Zhu et al., 2020). In addition to IL-6, also other factors including interferon γ, tumor necrosis factor (TNF), and Interleukin 1 (IL-1), etc., are over-produced during CRS and contribute to pathophysiological processes and multi-organ dysfunction (MOD) (Krishna et al., 2021). The cytokine storm (CS) during CRS may be brought under control by artemisinin or artesunate treatment as these block NF-κB signaling by inhibiting IKK activity (Efferte et al., 2002; Aldieri et al., 2003; Xu et al., 2007; Gu et al., 2012; Lai et al., 2015; Nunes et al., 2017; Efferte and Oesch, 2021). The activation of NF-κB signaling results in the downstream activation of the p50/p65 transcription factors, and artemisinin and artesunate may also act as an inhibitor in the NF-κB signaling pathway by blocking the function of p50/p65 in transcriptional activation of target genes like IL-6.

**ARTEMISININS: POTENTIAL MODES OF ACTION IN FIGHTING HUMAN CANCERS**

Artemisinins have been used to combat many different types of cancers and different modes of action have been described (Table 1). One of the most important mechanisms is preventing the activation of NF-κB signaling pathway involved in tumor induction, initiation, and progression of many cancerous cell lines. It is noteworthy that artemisinin may affect NF-κB signaling at different levels: it inhibits initiation of nuclear signaling by preventing interaction of p65 and p50 to cytosolic IKK, but in the nucleus it also inhibits interaction of p50 with target promoters (Tran et al., 2014) (see GRAPHICAL ABSTRACT). Also, Helicobacter pylori-induced gastric oncogenesis is inhibited by artemisinins through blocking NF-κB signaling. Remarkably in gastric cancer, artemisinins reverse the IkBα level, prevent NF-κB pathway in a dose-dependent manner, and decrease the generation of downstream inflammatory factors such as TNF-α (tumor necrosis factor-α) and IL-8 (interleukin-8) (Su et al., 2019). The artemisinin related compound dihydroartemisinin induces autophagy via suppressing NF-κB pathway in myeloma, colorectal, and cervical cancer cell lines (Hu et al., 2014), while the anti-invasive activity of dihydroartemisinin may occur through preventing of PKCa/Raf/ERK and JNK phosphorylation and decreasing NF-κB (Hwang et al., 2010). Indeed, increasing IkBα protein and blocking p65 subunit in NF-κB pathway is boosted by dihydroartemisinin (Dong et al., 2014).

Artesunate attenuates the growth of cancer cells and thus the development of the tumor by targeting NF-κB pathway (Table 1). In prostate cancer cells, resistance to androgen receptor antagonists is reduced by using artesunate. Mechanistically, the combination of artesunate and bicalutamide prevents NF-κB pathway by ubiquitin-mediated proteosomal deterioration (Nunes et al., 2017). In attempts to treat cervical cancer, the evidence demonstrated that artesunate successfully increases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity via pro-survival proteins including X-linked inhibitor of apoptosis protein (XIAP), survivin, and B-cell lymphoma-extra-large (Bcl-xL), and reduces the number of survival proteins in HeLa cells. The downregulation of mentioned proteins can be regulated by repressing activation of serine/threonine-protein kinase and NF-κB signaling. Artesunate further prevents TRAIL-influenced transcriptional activity of NF-κB (Thanaketpaisarn et al., 2011).

**ARTEMISININS: POTENTIALS IN COMBATING COVID-19 AND OTHER HUMAN VIRAL INFECTIONS**

**Physiological Effects of Artemisinins or Artemisia spp. Plant Extracts**

Currently, we are facing the serious challenge of the COVID-19 pandemic, which has disrupted global health and the economy. COVID-19 is a virus-related disease similar to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) (Liu et al., 2020) and is caused by the SARS-CoV-2 (Lai et al., 2020; Singhal, 2020). Unlike with SARS-CoV, patients infected with SARS-CoV-2 initially have mild symptoms and continue their daily activities, but in the meantime are infectious to others (Heymann and Shindo, 2020; Zheng et al., 2020). In some patients, disease symptoms may suddenly increase dramatically due to the development of a CS entitled CRS with hallmarks in the body of inflammation and immunosuppression (Mehta et al., 2020). CRS in COVID-19 patients may result in respiratory failures that create Acute Respiratory Distress Syndrome (ARDS) and MOD (Krishna et al., 2021). While worldwide efforts are aimed at vaccines that may prevent infection by COVID-19, additional medicines that can alleviate the severe symptoms of COVID-19 are still a high priority, also because new viral variants may escape vaccine recognition.

Previously, *in vitro* studies have indicated that the alkylating activity of activated artemisinin (as discussed above in the context of malaria) or the specific structure of artemisinin may have potential in preventing infections by members of the *Herpesviridae* family (e.g., herpes simplex virus type 1, Epstein-Barr virus, human cytomegalovirus), hepatitis B virus, hepatitis C virus, and bovine viral diarrhea virus (Efferte et al., 2008; Efferte, 2018). Artemisinin also has received renewed attention to fight emerging new viruses for which no effective antiviral drugs are available (e.g., HIV, dengue virus, chikungunya virus, Ebola virus), against viral strains that have developed drug resistance (e.g., human cytomegalovirus) and most recently against Coronavirus (D’alessandro et al., 2020). To combat infection by COVID-19, extracts from different medicinal plants (including from *Artemisia* spp.) have been tested against COVID-19 (Bahrami et al., 2020; Bailly and Vergoten, 2020; Huang et al., 2020;


| Artemisinins type | Type of cancer | Effects / Mechanism of action | References |
|------------------|----------------|-------------------------------|------------|
| Artemisinin      | Renal          | Inhibition of protein kinase B | Yu et al. (2019) |
| Artemisinin      | Breast         | Enhancing the anti-tumor immune response in 4T1 cancer cells | Cao et al. (2019) |
| Artemisinin      | Breast         | Inhibiting osteoclast formation | Li J. et al. (2019) |
| Artemisinin      | Breast         | Decreasing functional levels of estrogen receptor-alpha and ablating estrogen-induced proliferation | Sundar et al. (2008) |
| Artemisinin      | Breast         | Delaying the development of 7,12-dimethylbenz[a]anthracene (DMBA) | Lai and Singh (2006) |
| Artemisinin      | Breast         | Downregulating expression of the E2F1 transcription factor and loss of E2F1-target cell cycle genes | Tin et al. (2012) |
| Artemisinin      | Breast         | Reducing the number of regulatory T cells | Langroudi et al. (2010) |
| Artemisinin+Transferrin | Breast       | Retardation of cancer tumors | Lai et al. (2009) |
| Artemisinin      | Fibrosarcoma tumors | Inducing apoptosis in cancer cells | Singh and Lai (2004) |
| Artemisinin      | Ovarian        | Inducing reversal of EMT | Liang et al. (2019) |
| Artemisinin      | Prostate       | Blocks cancer growth and cell cycle progression by disrupting sp1 interactions with the cyclin-dependent kinase-4 (CDK4) promoter and inhibiting CDK4 gene expression | Willoughby et al. (2009) |
| Artemisinin      | Colon          | Inducing doxorubicin resistance in cancer cells via calcium-dependent activation of HIF-1α and P-glycoprotein | Riganti et al. (2009) |
| Artemisinin      | Cervical       | Repressing telomerase subunits and inducing apoptosis | Mondal and Chatterji (2015) |
| Artemisinin      | Ishikawa endometrial | Triggering a G1 cell cycle arrest of cancer cells, inhibiting cyclin dependent kinase-4 promoter activity and expression by disrupting NF-kB transcriptional signaling | Tran et al. (2014) |
| Artemisinin      | Neuroblastoma  | Reducing cell proliferation and inducing apoptosis | Zhu et al. (2014) |
| Artemisinin      | Nasopharyngeal | Down-regulation of BMI-1 cooperates | Wu J. et al. (2011) |
| Artemisinin      | Gastric        | Uregulation of p53 | Zhang et al. (2014) |
| Artemisinin      | Various Cancers | Inducing iron-dependent cell death (ferroptosis) in tumor cells | Ooko et al. (2015) |
| Artemisinin      | Various Cancers | Inhibition of tumor angiogenesis | Anfossi et al. (2006) |
| Artemisinin      | Colorectal     | Stimulating DR5-specific TRAIL-induced apoptosis by regulating wild type P53 | Zhou et al. (2003) |
| Artemisinin      | Gastric        | Inhibition of NF-κB signaling | Su et al. (2019) |
| Artemisinin+Hyperbaric Oxygen | Leukemia | Decreasing growth rate of cancer cells | Oghami et al. (2010) |
| 6-Aza-artemisin  | Various Cancers | In vitro cell-growth inhibitory activities | Koi et al. (2003) |
| Artemisinin+Estrogen | Breast and Cervical | Antiproliferative activity | Fröhlich et al. (2018) |
| Artemisinin+Artesunate dimer | Breast and Prostate | Inducing declines in proteins involved in apoptosis (survivin), cell cycling (cyclin D1), oncogenesis [c-myelocytomatosis oncogene product (c-MYC)], and dysregulated WNT signaling (beta-catenin) | Gong et al. (2013) |
| Artemisinin+Artesunate tagged Holotransferrin | Leukemia | Killing cancer cells | Lai et al. (2005) |
| Artemisinin+Artesunate | Lung | Elevating intracellular ROS and DNA damage | Li et al. (2018) |
| Artemisinin+Artesunate | Prostate | Induction of apoptosis | Nakase et al. (2009) |
| Artesunate       | Cancer stem cells | Mitochondrial dysfunction of stem cells | Subedi et al. (2016) |
| Artesunate       | Prostate       | Targeting NF-κappa B Signaling | Nunes et al. (2017) |
| Artesunate       | Prostate       | Suppressing the viability and mobility of cancer cells through UCA1, the sponge of miR-184 | Zhou et al. (2017) |
| Artesunate       | Head and Neck  | Inducing ferroptosis in cancer cells by decreasing cellular GSH levels, increasing lipid ROS levels, and activation of NF-κB-antioxidant response element pathway in cancer cells | Roh et al. (2017) |
| Artesunate       | HeLa cervical cancer cells | Mitochondrial fission, autophagy induction, and activating of the PINK1-dependent pathway | Zhang et al. (2018) |
| Artesunate       | Cervical       | Inhibiting PGE2 production and Foxp3 expression | Zhang et al. (2014) |
| Artesunate       | Cervical       | Inducing radiosensitivity | Luo et al. (2014) |
| Artesunate       | Cervical       | Enhancing TRAIL-induced apoptosis in cancer cells through inhibition of the NF-κB and PI3K/Akt signaling pathways | Thanakatpaisarn et al. (2011) |
| Artesunate       | Colorectal     | Reducing K67 and increasing CD31 expression | Krishna et al. (2015) |
| Artesunate       | Colorectal     | Down-regulating immunosupression from Colon26 and RKO cells by decreasing transforming growth factor β1 and interleukin-10 | Cui et al. (2015) |
| Artesunate       | Colorectal     | Expression of beta-catenin and E-cadherin | Li et al. (2008) |
| Artesunate       | Colorectal     | Attenuating the growth of cancer cells and inhibiting hyperactive Wnt/b-catenin pathway | Li et al. (2007) |
| Artesunate       | Colorectal     | Suppressing inflammation and oxidative stress | Kumar et al. (2019) |
| Artesunate       | Colorectal     | Activating the intrinsic apoptosis of HCT116 cells through the suppression of fatty acid synthase and the NF-κB Pathway | Chen et al. (2017) |
| Artemisinins type | Type of cancer | Effects / Mechanism of action                                                                 | References               |
|------------------|---------------|-----------------------------------------------------------------------------------------------|--------------------------|
| Artesunate       | Colorectal    | Down-regulating β-catenin, suppressing of angiogenesis, cellular proliferating and inducing of apoptosis | Verma et al. (2017)      |
| Artesunate       | Bladder       | Inducing autophagy dependent apoptosis through upregulating ROS and activating AMPK-mTOR-ULK1 axis | Zhou et al., 2020a       |
| Artesunate       | Leukemia      | Inhibiting angogenesis and down-regulating vascular endothelial growth factor expression      | Zhou et al. (2007)       |
| Artesunate       | T-cell leukemia/lymphoma | Increasing of intracellular ROS and activation of the DNA damage marker γ-H2AX             | Ishikawa et al. (2020)  |
| Artesunate       | Skin          | Induction of G0/G1 cell cycle arrest and iron-mediated mitochondrial apoptosis                | Jiang et al. (2012)     |
| Artesunate       | Liver         | Inducing G0/G1 cell cycle arrest and apoptosis via increasing intracellular ROS               | Yin et al. (2020)       |
| Artesunate       | Liver         | Mitigating proliferation of tumor cells by alkylating heme-harboring nitric oxide synthase   | Zeng and Zhang (2011)   |
| Artesunate       | Laryngeal     | Reducing of tumor proliferation                                                                | Singh and Verma (2002)   |
| Artesunate       | Ovarian       | Promoting Th1 differentiation from CD4+ T cells to enhance cell apoptosis via miR-142        | Chen et al. (2019)       |
| Artesunate       | Ovarian       | Sensitizing cancer cells to cisplatin by downregulating RAD51                                 | Wang B. et al. (2015)    |
| Artesunate       | Ovarian       | Inhibiting cancer cell growth and proliferation                                                | Greenshields et al. (2017)|
| Artesunate       | Ovarian       | Reducing cell viability                                                                        | McDowell et al. (2021)  |
| Artesunate       | Ovarian       | Inducing oxidative DNA damage, sustaining DNA double-strand breaks, and the ATM/ATR damage response | Berdelé et al. (2011)    |
| Artesunate       | Glioblastoma  | Inducing ROS and p38 MAPK-mediated apoptosis and counteracting tumor growth                    | Beccacico et al. (2015)  |
| Artesunate       | Merkel cell carcinoma | Affecting T antigen expression and repressing growth and survival of MCPyV-positive cancer cells | Sarma et al. (2020)     |
| Artesunate       | Breast        | Inhibition of the growth of MCF-7 tumor cell                                                 | Dong and Wang (2014)     |
| Artesunate       | Breast        | Inducing apoptosis pathway by loading into lipid carriers                                      | Tran et al. (2016)       |
| Artesunate       | Breast        | Induction of apoptosis                                                                         | Jamatzadeh et al. (2017) |
| Artesunate       | Breast        | Enhancing the efficacy of 5-ALA-based SDT                                                      | Osaki et al. (2017)      |
| Artesunate       | Breast        | Activating mitochondrial apoptosis in cancer cells via iron-catalyzed lysosomal ROS production | Hamacher-Brady et al. (2011)|
| Artesunate       | Breast        | Inducing G2/M cell cycle arrest through autophagy induction                                   | Chen et al. (2014)       |
| Artesunate       | Breast        | Down-regulating the expression of Bcl-2 and HSP70, Enhancing the expression of cleaved caspase-9 in MCF-7 and 4T1 cells | Pirali et al. (2023)     |
| Artesunate       | Breast        | Promoting G2/M cell cycle arrest in MCF7 cancer cells through ATM activation                  | Wen et al. (2018)        |
| Artesunate       | Endometrial   | Suppressing the proliferation and development of estrogen receptor-α-positive in HAND2-dependent pathway | Yin et al. (2021)       |
| Artesunate       | Lung          | Inhibiting invasion and in vivo metastasis in cancer cells by targeting essential extracellular proteases | Rasheed et al. (2010)    |
| Artesunate       | Lung          | Expression of EGFR and ABCG2                                                                 | Ma et al. (2011)         |
| Artesunate       | Bladder       | Inducing apoptosis of cancer cells by miR-16 regulation of COX-2 expression                   | Zuo et al. (2014)        |
| Artesunate       | Bladder       | Impairing growth in cisplatin-resistant cancer cells by cell cycle arrest, apoptosis and autophagy induction | Zhao et al. (2020)       |
| Artesunate       | Colon         | Enhancing ablation effect on xenograft cancer cells                                           | Hao et al. (2020)        |
| Artesunate       | Colon         | Inducing apoptosis and autophagy                                                              | Jiang et al. (2018)      |
| Artesunate       | Nitrosodiethylamine mediated experimental hepatocellular model | Suppression of IL-6-JAK-STAT signaling                                                       | Ilamathi et al. (2016)  |
| Artesunate       | HeLa and HepG2 cells | Inducing cell death in cancer cells via enhancing lysosomal function and lysosomal degradation of ferritin | Yang et al. (2014)       |
| Artesunate       | Non-small-cell lung | Inhibiting epithelial-mesenchymal transition in cancer cells by down-regulating the expression of BTBD7 | Wang et al. (2020)       |
| Artesunate       | Non-small cell lung | Enhancing radiosensitivity cancer cells via increasing NO production to induce cell cycle arrest at G2/M phase | Zhao et al. (2011)       |
| Artesunate       | Pancreatic     | Inducing AsPC-1 and PaTu8988 cell death                                                        | Wang K. et al. (2019)    |
| Artesunate       | Pancreatic     | Activating of ferroptosis                                                                      | Eling et al. (2015)      |
| Artesunate       | Gastric        | Inhibiting the growth of cancer cells through the mechanism of promoting oncosis               | Zhou et al. (2013)       |
| Artesunate       | Gastric        | Inhibiting cancer cell growth and inducing apoptosis by down-regulating COX-2               | Zhang et al. (2015)      |
| Artesunate       | B-cell lymphoma | Suppressing cancer cell growth and metabolism                                                   | Våtsveen et al. (2018)   |
| Artemisinins type | Type of cancer | Effects / Mechanism of action | References |
|------------------|---------------|-------------------------------|------------|
| Artemisinin      | Bone metastasis | Suppressing RANKL-induced osteoclastogenesis through inhibition of PLCy1-Ca²⁺ - NFATc1 signaling pathway and preventing ovariectomy-induced bone loss | Zeng et al. (2017) |
| Artemisinin      | Esophageal     | Cell apoptosis and suppressing the proliferation | Shih et al. (2015) |
| Artemisinin      | Esophageal     | Enhancing radiosensitivity of cancer cells by inhibiting the repair of DNA damage | Fei et al. (2018) |
| Artemisinin      | Dermal fibroblasts | Inhibiting myofibroblast formation via induction of apoptosis and antagonism of pro-fibrotic gene expression | Larson et al. (2019) |
| Artemisinin+Histone Deacetylase Inhibitors | Hepatocellular, Colorectal, Lung, and Pancreatic | Elevating heme synthesis via synergistic upregulation of ALAS1 expression | Chen et al. (2019) |
| Artemisinin+Ferrous iron | Leukemia and Astrocytoma | Induction of apoptosis | Effert et al. (2004) |
| Artemisinin+ Sorafenib | Liver | Inhibiting cancer cell growth and apoptosis induction | Li et al. (2019) |
| Artemisinin+ Cisplatin | Lung | Inhibiting MAPK pathway | Li et al. (2021) |
| Artemisinin and Dihydroartemisin | Neuroblastoma | Inducing apoptosis and ROS in cancer cells | Michaelis et al. (2010) |
| Artemisinin+ Concoxin-43 | Renal and Breast | DNA damage and enhancing the bystander apoptosis of the neighboring cells | Raza et al. (2017) |
| Artemisinin+ Allicin | Osteosarcoma | Inhibiting cell proliferation and apoptosis | Jiang et al. (2013) |
| Artemisinin and Dihydroartemisin | Epithelial ovarian | Inhibiting epithelial ovarian cancer cells via autophagy-mediated cell cycle arrest and suppressing the cell cycle-related NF-κB-signaling pathway | Li et al. (2018) |
| Dihydroartemisin | Colorectal | Potentiation of 5-fluorouracil antitumor activity | Yao et al. (2018) |
| Dihydroartemisin | Colorectal | Induction of iron-dependent endoplasmic reticulum stress | Lu et al. (2011) |
| Dihydroartemisin | HeLa cervical cancer cells | Autophagy within cancer cells through Bcl-2 phosphorylation at Ser70 | Wang et al. (2019) |
| Dihydroartemisin | Cervical | Cytotoxic activity against papillomavirus-expressing epithelial cells | Disbrow et al. (2005) |
| Dihydroartemisin | Esophageal | Inactivating of NF-κB in Eca109 and Ec9706 | Li et al. (2014) |
| Dihydroartemisin | Esophageal | Increasing the sensitivity of photodynamic therapy via NF-κB/HIF-1α/VEGF pathway | Li et al. (2018) |
| Dihydroartemisin | Breast | Inducing apoptosis | Mao et al. (2013) |
| Dihydroartemisin | Hepatocellular | Inhibiting proliferation and inducing apoptosis of cancer cell by upregulating tumor necrosis factor via JNK/NF-κB pathways | Wu et al. (2019) |
| Dihydroartemisin | Ovarian | Inducing apoptosis and inhibiting proliferation, migration, and invasion in cancer cells via inhibition of the hedgehog signaling pathway | Liu et al. (2018) |
| Dihydroartemisin | Ovarian | Inhibiting PDGF-Rα-positive cancer cell growth and metastasis through inducing degradation of PDGF-Rα protein | Li et al. (2017) |
| Dihydroartemisin | Ovarian | Inhibiting cancer cell growth, inducing apoptosis and G2 cell cycle arrest, decreasing of Bcl-xL and Bcl-2, and increasing of Bax and Bad | Jiao et al. (2007) |
| Dihydroartemisin | Various Cancers | Inhibiting angiogenesis | Chen et al. (2003) |
| Dihydroartemisin | Cholangiocarcinoma and Hepatocarcinoma | Expression of TDR1, MDR1, MRP1, MRP2, and MRP3 | Chaijaroenkul et al. (2011) |
| Dihydroartemisin | Pancreatic | Inhibiting cell viability, downregulating the expression of proliferating cell nuclear antigen and cyclin D1, upregulated p21WAF1/CIP1, inducing apoptosis by reducing the ratio of Bcl-2/Bax and increasing the activation of caspase-9 | Chen et al. (2009) |
| Dihydroartemisin | Pancreatic | Inducing oncasis-like cell death | Du et al. (2010) |
| Dihydroartemisin | Pancreatic | Inducing cell cycle arrest, apoptosis, and inhibiting of NF-κB signaling | Chen et al. (2010) |
| Dihydroartemisin | Pancreatic | Inducing NF-κB pathway | Wang et al. (2011); Wang et al. (2012) |
| Dihydroartemisin | Non-small-cell lung | Suppressing metastasis of cancer via inhibiting NF-κB/GLUT1 axis | Jiang et al. (2016) |
| Artemisone | Melanoma | Inhibiting cancer cell growth | Dwivedi et al. (2015) |
| Artemisone | Breast, Colon, Melanoma, and Pancreatic | Reducing cell viability and arresting cell cycling | Gravett et al. (2011) |
| Artesunate | Gastric | Increasing of DNA-damage index, inducing necrosis in PG100, inducing both apoptosis and necrosis in lymphocytes | Acián et al. (2013) |
| Artemisinic acid+Thymoquinone | Colorectal | Increasing of ROS, and elevating levels of DNA-damage marker γ-H2AX | Fröhlich et al. (2017) |
| Anhydro dihydroartemisin and 10-dihydroartemisinyl acetate | Liver/Colon | Antiproliferative and inhibiting the release of BVDV-RNA | Blazquez et al. (2013) |
| Artemisinin, Dihydroartemisin, and Artesunate | Non-small-cell lung | Inhibiting tumorigenesis and tumor metastasis through Wnt/β-catenin signaling | Tong et al. (2016) |
Kapepula et al., 2020; Koshak and Koshak, 2020; Mani et al., 2020; Verma et al., 2020; Williamson and Kerimi, 2020; Belhassan et al., 2021; Hassanipour et al., 2021; Javed et al., 2021; Lyu et al., 2021; Nazrul Islam et al., 2021; Song et al., 2021).

In vitro efficacy of artemisinin-based treatments to combating SARS-CoV-2 has indicated that treatment with artesunate, artemether, *A. annua* extracts, and artemisinin hindered virus infections of human lung cancer A549-hACE2 cells, VeroE6 cells, and human hepatoma HuH7.5 cells. Among these four treatments, artesunate showed the strongest anti-SARS-CoV-2 activity (7–12 µg/mL), followed by artemether (53–98 µg/mL), *A. annua* extracts (83–260 µg/mL), and artemisinin (151 to at least 208 µg/mL). Collectively, time-of-addition experiments in A549-hACE2 cells displayed that artesunate attacked the virus at the post-entry level (Zhou et al., 2021). In parallel with the previous study, dried-leaf hot-water extracts of *A. annua* cultivars including SAM, BUR, A3, and MED revealed in vitro anti-SARS-CoV-2 activity against Alpha, Beta, Gamma, Delta, and Kappa variants of the virus. All cultivars in addition to being potent in combating with original wild type WA1 also showed effective potential against mentioned variants. IC90 and IC50 according to measured artemisinin content ranged from 1.4–25.0 µM and 0.3 to 8.4 µM, respectively. Also, the IC90 and IC50 according to dried-leaf weight ranged from 59.5–160.6 µg DW and 11.0 to 67.7 µg DW, respectively (Nair et al., 2022). Alternatively, *Artemisia* spp. Extracts and COVID-Organics drink produced in Madagascar hindered in vitro SARS-CoV-2 and Feline coronavirus (FcoV) infections at concentrations that did not influence cell efficacy and viability (Nie et al., 2021).

In another successful in vitro study, six monomer compounds including artesunate, artemether, arteannuin B, andrographolide, licochalcone B, and echinatin exerted high anti-SARS-CoV-2 and anti-GX_P2V (pangolin coronavirus) activity (Hu et al., 2021). In addition to the mentioned monomers, it is noteworthy that the quinoline like artemisinins has shown strong anti-SARS-CoV-2 activity (Firestone et al., 2021). Also, the anti-SARS-CoV-2 activity of nine artemisinin-based compounds experimented in vitro. Results highlighted that arteannuin B, artesunate, and dihydroartemisinin are the most potent agents in inhibiting virus activity. Also, several artemisinins decreased the generating of the virus nucleocapsid (N) proteins in a dose-dependent manner. It can be concluded that targeting N proteins can be considered as one of the possible options to control viral infection. On the other hand, both lumeferantrine and arteannuin B suppressed viral infection following SARS-CoV-2 entry toward the host cells (Cao et al., 2020). In addition to artemisinins monotherapy, in vitro inhibition of SARS-CoV-2 entry toward the host cells (Cao et al., 2020). In addition to artemisinins monotherapy, in vitro inhibition of SARS-CoV-2 replication by artemisinin-based combination therapies (ACTs) in African experimental society indicated that the artesunate-mefloquine exerted high anti-SARS-CoV-2 activity with % inhibition of 72.1 ± 18.3%. Also, other ACTs including artesunate-pyronaridine, artesunate-amodiaquine, dihydroartemisinin-piperaquine, and artemether-lumeferantrine displayed the same range of inhibition (27.1 to 34.1 %) (Gendrot et al., 2020). Along with in vitro studies of ACTs, molecular docking studies have also demonstrated the anti-SARS-CoV-2 activity of artemisinin-thymoquinone hybrids against the main protease of the virus (de Oliveira et al., 2021).

**Molecular Modes of Action of Artemisinins in Combating COVID-19**

**Blocking Receptor Binding of Spike Protein to Host Cell Surface**

While extracts from medicinal plants may show some preliminary efficacy in small scale clinical trials (Dong et al., 2020; reviewed in Oregi et al., 2021), these do not clarify where the activity is coming from. For instance, the antiviral and immunomodulation effects of *Artemisia* spp. Extracts, as recently been reviewed (Kshirsagar and Rao, 2021), may not only be due to artemisinins, but also from other potential bioactive compounds like flavonoids, mono- and sesqui-terpenes or tannins in these extracts (Kshirsagar and Rao, 2021). To address efficiency and potential harmful side effects of plant extracts, a more detailed knowledge on the molecular mode of action of individual bioactive molecules is needed (Cheong et al., 2020; Guastalegname and Vallone, 2020). Studies indicate that artesunate, dihydroartemisinin, and artemisinin may act at the cell surface by inhibition of the binding of the SARS-CoV-2 spike protein to cell surface receptors, thus potentially preventing both endocytosis of the virus and activation of the NF-κB signaling pathway (Gendrot et al., 2020; Rolta et al., 2020; Sehailia and Chemat, 2020; Uckun et al., 2021).

However, molecular docking studies indicate that artemisinins may also bind to coronavirus-host proteins such as E protein, helicase protein, N protein, 3CL<sup>PRO</sup>, S protein, nonstructural protein 3 (nsp3), nsp10, nsp14, nsp15, cathepsin-L, and glucose-regulated protein 78 receptor (Fuzimoto, 2021; Ribaudo et al., 2021) and part of the biological activity of artemisinin against COVID-19 may thus also be partially based on inhibiting the function of these viral proteins.

**Preventing Cytokine Storm by Inhibiting IKK**

In addition, artemisinin/and or artesunate may limit CS by inhibiting IKK and thus over-active NF-κB signaling, or it may inhibit the transcriptional activity of p50/p65, released by NF-κB signaling (see GRAPHICAL ABSTRACT). While preliminary studies with artemisinins look promising, researchers have warned that the potential of artemisinins in combating COVID-19 may thus also be partially based on inhibiting the function of these viral proteins.

**Artemisinins-Related Clinical Trials in Combating COVID-19**

A total of 16 trials with *Artemisia* spp. Extract, artemisinins, and ACTs have been registered in the US National Library of Medicine<sup>1</sup> with clinical trial IDs: NCT04530617, NCT04306497, NCT04701606, NCT04695197, NCT04475107, NCT04532931, NCT04374019, NCT05084911, NCT04502342, NCT04801017, NCT04374084, NCT05004753, NCT04387240, NCT04553705, NCT04802382, NCT04382040, and 3 trials in Chinese Clinical Trial Registry (ChiCTR) database<sup>2</sup> with IDs: ChiCTR2000033049, ChiCTR2000032915, and ChiCTR2000033049.

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<sup>1</sup><https://clinicaltrials.gov/

<sup>2</sup><https://www.chictr.org.cn/>
Until December 2021, only one trial registered in https://clinicaltrials.gov/ has been terminated with ID number: NCT04530617. Preliminary results from this study indicate that the agents such as *A. annua* and Camostat mesilate may help reduce the number of hospitalized patients and that the use of artemisinin-piperaquine for treatment of COVID-19 is safe (Li et al., 2020). Therefore, the World Health Organization (WHO) has initiated clinical trials on three promising candidate drugs, including artesunate, to evaluate the anti-inflammatory activity against SARS-CoV-2 (Solidarity Trial PLUS is registered at: ISRCTN83971151). As new mutations occur in the SARS-CoV-2, resulting in new variants including Alpha, Beta, Gamma, Delta, Kappa, and Omicron, combating potential downstream effects of COVID-19 infections remains an important aspect of dealing with the ongoing pandemic, especially when this virus has become endemic.

**NATURAL ARTEMISININ PRODUCTION: LOW YIELD AND ALTERNATIVES TO BOOST PRODUCTION**

Artemisinin is produced in glandular trichomes in the leaves and ovary of the *A. annua* (Wang et al., 2016). Both the specificity of artemisinin biosynthesis occurring in GTs and the fact that GTs represent about 2% of plant total weight put a
limit to the bulk production of artemisinin in planta (Judd et al., 2019). The different steps in artemisinin production are shown in Figure 3. The end-product of the enzymatic pathway (dihydroartemisinic acid) is presumably toxic to the plant cell and is therefore exported over the plasma membrane and the cell wall to a subcuticular space where it is converted non-enzymatically by light (UV) to artemisinin (Wang et al., 2016). In parallel, artemisinic acid, another end-product of the enzymatic pathway may also be exported from the cell, and extracellularly converted to arteannuin B. Typical yield of artemisinin from the Artemisia annua plant is 0.6 to 1.2% but may go up to 2% based on plant dry weight (Zhang et al., 2008). However, such yield is low and far from the potential world’s demand (Judd et al., 2019). Current supplies of artemisinin are already limiting to treat people for malaria in a cost-effective way, so use in treating the disease of pandemic proportions like COVID-19 will need new approaches to artemisinin production. Several methods have been proposed to increase artemisinin production so far which are treatments impacting A. annua cultivation and physiology and breeding approaches to increase artemisinin yield, engineering artemisinin biosynthetic and transport pathway in the native A. annua plants, and engineering of heterologous (plant and microorganism) systems via ectopic expression of the biosynthetic pathway (Lei et al., 2011; Liu et al., 2011; Parshikov et al., 2012; Fuentes et al., 2016; Kiani et al., 2016; Ikram and Simonsen, 2017; Carqueijeiro et al., 2020).

**TREATMENTS IMPACTING ARTEMISININ CONTENT in Artemisia annua**

Thanks to targeted breeding programs different cultivars of A. annua can be grown in a wide range of climate conditions (temperate, cold temperate, subtropical, and Mediterranean) (Ferreira et al., 2005). However, the content and composition of secondary metabolites in A. annua plants are determined by numerous interacting factors: geographical conditions, harvesting time, agricultural practices (e.g., fertilization, irrigation, density per unit area) and post-harvest conditions (Mert et al., 2002; Jelodar et al., 2014). Conventional manipulations of the A. annua plant that may enhance artemisinin production are discussed below and summarized in Figure 4.

**Nutrient Manipulations**

In addition to genetic factors, the artemisinin yield can be influenced by environmental conditions and field management practices (Charles et al., 1991). Furthermore, the use of fertilizer compounds can also affect the artemisinin content. As studies have shown, manure and chemical fertilizers are effective in the production of secondary metabolites by improving the photosynthetic rate and carbon production (Jha et al., 2011). Numerous reports have considered the use of nitrogen (N) fertilizer to be somewhat effective in increasing the artemisinin content (Ferreira et al., 2005; Davies et al., 2009; Aftab et al., 2011). In addition to the important role of macronutrients in increasing the artemisinin production in Artemisia plants, studies showed that the deficiency of micronutrients (iron, copper, zinc, and barium) also plays a significant role in reducing the artemisinin content (Srivastava and Sharma, 1990).

**Biotic Elicitors**

Similarly, the use of elicitors in plants increases the accumulation of secondary metabolites (Zhao et al., 2005). For instance, the elicitor of Penicillium chrysogenum extract has increased (up to double) the production of artemisinin in hairy roots of A. annua (Liu et al., 1999). Additionally, the application of chitosan or of the arbuscular mycorrhizal species Rhizophagus intraradices (as elicitor) was able to increase the content of dihydroartemisinic acid and artemisinin in Artemisia plants (Lei et al., 2011; Mandal et al., 2014). Since the response to these biotic factors often involves the phytohormone jasmonic acid (JA), the effect of these factors on artemisinin content could be due to activated JA signaling.

**Plant Breeding**

The main breeding goals of A. annua are the improvement of artemisinin production by increasing the yield potential of leaves, proliferate the number of shoots, and raising the total number of glandular trichomes per plant (Graham et al., 2010; Jelodar et al., 2014). Selection through germplasm and genetic modification can be considered as basic strategies for the improvement of artemisinin production in A. annua (Charles et al., 1991; Xie et al., 2016). A very common breeding technique to increase the secondary metabolites is the manipulation of the ploidy levels in plants (Weathers, 2003). The production of artificial polyploids as a plant breeding strategy has made it possible to develop new and improved cultivars (Iannicelli et al., 2020). In this regard, the application of ploidy manipulation techniques has successfully increased the artemisinin production in A. annua. Reports indicate that the amount of artemisinin in tetraploid plants has increased up to 56% compared to diploid plants. Alternatively, induced mutation using chemicals such as sodium azide (NaN₃) and ethyl methane sulfonate (EMS) was effective in increasing the artemisinin biosynthesis in native plant (Al-Qurainy and Khan, 2010; Leow et al., 2020).

**Enhancing Glandular Trichomes**

In some plant species, the production of some glandular type trichomes is enhanced by JA treatment (Chen et al., 2018) or UVB light (Yan et al., 2012). Indeed, the artemisinin content of A. annua is enhanced under the UV treatment at a dosage of 150 gray irradiation (Raymond et al., 2015) and UV-B radiation at 1.44 kJ m⁻² d⁻¹ (Pandey and Pandey-Rai, 2014) respectively. This could be due to both an effect on glandular trichome density and enhanced conversion of artemisinic aldehyde to artemisinin.

**Plant Growth Regulators**

Various agricultural practices use plant growth regulators (PGRs) to improve artemisinin production. For example, the treatment of A. annua with Salicylic acid increases plant growth, leading to higher biomass (Aftab et al., 2010), altered plant morphology, artemisinin content and composition (Ma et al., 2009). Other
studies have shown that PGR GA$_3$ (Weathers et al., 2005; Zhang et al., 2005; Aftab et al., 2011) and JA can also increase the artemisinin content of $A$. annua (Zhou and Memelink, 2016). JA has been shown to boost artemisinin biosynthesis via the releasing of repressors of transcription factor TCP14-ORA at the promoters of double bond reductase 2 (DBR2) and aldehyde dehydrogenase 1 (ADH1), two key genes in the artemisinin biosynthetic pathway (see Figure 3; Ma et al., 2018).

**BIOENGINEERING OF ARTEMISININ PRODUCTION IN Artemisia annua**

Metabolic engineering may be used to improve the production of artemisinin in $A$. annua itself but is hampered by the difficulties in efficient transformation and regeneration of $A$. annua plants. Alternatively, the genes that have been isolated from $A$. annua that are involved in artemisinin production may be expressed in...
TABLE 2 | Introducing artemisinin (ART) pathway genes in Artemisia annua L. to improve the ART production using different strategies.

| Expression type                        | Yield            | References                     |
|----------------------------------------|------------------|--------------------------------|
| Artemisia annua L. Overexpression      | 1.73 mg/g DW     | Alam and Abdin (2011)          |
| of HMGR and ADS                         |                  |                                |
| Artemisia annua L. Overexpression      | 0.98 ± 0.18 mg/g | Shen et al. (2012)             |
| of CYP71AV1 and CPR                    |                  |                                |
| Artemisia annua L. Overexpression      | 2.9 mg/g FW      | Chen et al. (2013)             |
| of FPS, CYP71AV1 and CPR               |                  |                                |
| Artemisia annua L. Overexpression      | 1.3% DW          | Banyai et al. (2010)           |
| of FPS                                 |                  |                                |
| Artemisia annua L. Overexpression      | 0.386 ± 0.0332mg/g DW | Aquil et al. (2009)        |
| of HMGR                                 |                  |                                |
| Artemisia annua L. Suppressing the    | 31.4 mg/g DW     | Zhang et al. (2009)            |
| expression of SQS                       |                  |                                |
| Artemisia annua L. Overexpression      | ≥ 14 4 mg/g DW   | Jiang W. et al. (2016)         |
| of AaWRKY1                              |                  |                                |
| Artemisia annua L. Overexpression      | 1.5–2.14 mg/g DW | Yuan et al. (2015)             |
| of DBR2                                 |                  |                                |

TABLE 3 | Introducing artemisinin (ART) pathway genes in planta to improve the ART production using different strategies.

| Expression type                        | Yield            | References                     |
|----------------------------------------|------------------|--------------------------------|
| Nicotiana benthamiana Transient       | 0.000220347 mg/g FW | Ting et al. (2013)             |
| expression of ART precursors' genes    |                  |                                |
| Nicotiana benthamiana Expression of   | 2e-7-1.7e-6mg/g FW | Wallaart et al. (2001)         |
| ADS                                    |                  |                                |
| Nicotiana benthamiana Stable          | 0.00048-0.00094 mg ART/g DW | Farihi et al. (2011)        |
| transformation of ADS                  |                  |                                |
| Nicotiana benthamiana Stable          | 0.005-0.0068 mg ART/g DW | Farihi et al. (2011)         |
| transformation of mtADS                |                  |                                |
| Nicotiana benthamiana Stable          | AD: > 0.004 mg/g FW; AA: > 0.0005 mg/g FW; DA: > 0.0015 mg/g FW | Zhang et al. (2011)          |
| transformation of ADS, CYP71AV1, and DBR2 |                  |                                |
| Nicotiana benthamiana Transient       | 0.003 mg/g DW     | Wang et al. (2016)             |
| expression of AaLTP3 and AaPDR2        |                  |                                |
| Nicotiana benthamiana SPG Transformation | > 0.12 mg artemisinic acid/g biomass | Fuentes et al. (2016)       |
| Nicotiana benthamiana Stable          | 0.3–0.8 mg/g DW   | Mahotra et al. (2016)          |
| transformation of ART B.P. genes       |                  |                                |
| Nicotiana benthamiana Stable          | 0.0395 mg/g FW    | van Herpen et al. (2010)       |
| transformation of ART B.P. genes       |                  |                                |
| Physcomitrella patens Stable          | 0.21 mg/g DW      | Ikram et al. (2017)            |
| transformation of ART B.P. genes       |                  |                                |

SPG, Stable Plastid Genome; FW, Fresh Weight; DW, Dry Weight; AD, Amorphadiene; AA, Artemisinic alcohol; DA, Dihydroartemisinic alcohol; ART B.P., Artemisinin biosynthetic pathway. All units are converted to milligram per gram (mg/g).

FIGURE 6 | Heterologous overexpression of genes from artemisinin biosynthetic pathway in the host plants N. benthamiana and P. patens.

a heterologous host that is easier to transform and grow (Covello, 2008; Ma et al., 2015; Xie et al., 2016; Lv et al., 2017; Ikram et al., 2019). Options to manipulate artemisinin production in A. annua are briefly discussed below and summarized in Figure 5. The yield effects of the different transformation efforts of A. annua are summarized in Table 2.

(1) Artemisia annua has been transformed with Agrobacterium genes that affect endogenous plant hormone levels (rol ABC or ipt), resulting in mild to up to 9 times higher artemisinin levels compared to untransformed (or empty vector transformed) control plants (Sa et al., 2001; Bulgakov, 2008; Dilshad et al., 2015; Kiani et al., 2016).

(2) Other transformation strategies are aimed at boosting precursors, either by boosting flux through the Mevalonate pathway by ectopic expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) or
TABLE 4 | De novo production of ART precursor via synthetic biology.

| No. | Host                        | Gene(s)                                                                                                                                 | Yield                                                                 | References               |
|-----|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------|
| 1   | Saccharomyces cerevisiae    | Amorpha-4,11-diene synthase                                                                                                                                                                    | Plasmid and genome-transformed produced 0.6 and 0.1 mg/l amorphadiene | Lindahl et al. (2006)    |
| 2   | Saccharomyces cerevisiae    | Mevalonate pathway, amorphadiene synthase, cytochrome P450 monoxygenase                                                                  | ≥ 100 mg/l amarvisinic acid                                           | Ro et al. (2006)         |
| 3   | Saccharomyces cerevisiae    | Amorphadiene synthase, amorphadiene oxidase, and cytochrome P450 reductase                                                            | 250 mg/l (in shake-flask) and 1000 mg/l (in bioreactors) artemisinic acid | Ro et al. (2008)         |
| 4   | Saccharomyces cerevisiae    | Mevalonate pathway, overexpression of related genes                                                                                   | > 40000 mg/l amorphadiene                                            | Westfall et al. (2012)   |
| 5   | Saccharomyces cerevisiae    | Complete biosynthetic pathway                                                                                                               | 250000 mg/l amarvisinic acid                                        | Paddon et al. (2013)     |
| 6   | Escherichia coli            | Expression of a synthetic amorpha-4,11-diene synthase and the mevalonate isoprenoid pathway from Saccharomyces cerevisiae               | 24 mg caryophyllene equivalent/l amorphadiene                        | Martin et al. (2003)     |
| 7   | Escherichia coli            | Nine genes from mevalonate pathway                                                                                                       | 500 mg/l amorphadiene                                               | Newman et al. (2006)     |
| 8   | Escherichia coli            | Overexpression of mevalonate pathway genes                                                                                               | > 25000 mg/l amorphadiene                                           | Tsuruta et al. (2009)    |
| 9   | Escherichia coli            | Amorphadiene biosynthetic pathway genes                                                                                                 | 293 mg/l/OD<sub>600</sub> at 75h amorphadiene                      | Anthony et al. (2009)    |
| 10  | Escherichia coli            | Engineered substrate promiscuous P450<sub>BM3</sub>                                                                                            | 250 mg/l amorphadiene                                               | Dietrich et al. (2009)   |
| 11  | Escherichia coli            | Mevalonate pathway genes                                                                                                                  | 235 mg/l amorphadiene                                               | Wu T. et al. (2011)      |

All units are converted to milligram per liter (mg/l).

by blocking unwanted side reactions that drain from the precursor pool (e.g., Squalene synthase (SQS), that diverts FPP to squalene) (Liao et al., 2016). Ectopic expression of HMGR in A. annua can boost artemisinin production (Aquil et al., 2009; Nafis et al., 2011; Ma et al., 2017), while also suppression of SQS can increase artemisinin levels (Paradise et al., 2008; Yang et al., 2008; Zhang et al., 2009; Table 2 and Figure 5).

(3) Transformation approaches may also be aimed at boosting flux through the artemisinin biosynthetic pathway itself through overexpression of biosynthesis genes. Ectopic overexpression of farnesyl pyrophosphate synthase (FPS) alone or FPS with CYP71AV1 and CPR, increased artemisinin levels in transgenic plants (Table 2; Chen et al., 2000, 2013; Han et al., 2016; Banyai et al., 2010; Wani et al., 2021). In another study, upregulated expression of HMGR, FPS, ADS, Aldh1, and ADS in A. annua increased artemisinin level 39-56% fold (Lin et al., 2011; Figure 5).

(4) The expression of endogenous biosynthesis genes may also be boosted by ectopic overexpression of relevant transcription factors (TF), provided expression of such TF is limiting for transcription of target genes. Multiple TFs (AP2/ERFs, WRKYs, bHLH, MYCs) have been identified in the regulation of endogenous artemisinin biosynthesis genes (Verpoorte and Memelink, 2002; Yang et al., 2012; Shen et al., 2016; Lv et al., 2017), but not all of these have been tested for stable transformation of A. annua. However, ectopic expression of WRKY does result in higher artemisinin production in A. annua (Jiang W. et al., 2016; Table 2 and Figure 5).

(5) The capacity for extracellular accumulation of dihydroartemisinic acid for extra-cellular conversion to dihydroartemisin may be of importance for the flux through the biosynthetic pathway to prevent feedback inhibition and possible toxic effects of pathway products. Studies in tobacco have shown that ABC-transporter AaPDR2, in concert with specific LTP AaTLP3 may be required for this function (Wang et al., 2016). In the tobacco assay, AaTLP3 and AaPDR2 prevent dihydroartemisinic acid reflux from the apoplast to the cell, resulting in higher artemisinin levels (Wang et al., 2016). However, manipulation of either ABC-transporter or LTP levels in A. annua has not been performed till now. Potentially, overexpression of these proteins could result in enhanced artemisinin production in A. annua (Figure 5).

**BIOENGINEERING OF ARTEMISININ IN HETEROLOGOUS PRODUCTION PLATFORMS**

**In planta Artemisinin Production**

The genes for artemisinin production have also been expressed in other plants, either by transient expression or by stable
transformation. To date, in planta artemisinin production has been reported for tobacco (Nicotiana benthamiana) and moss (Physcomitrella patens) (Table 3). Transient expression of genes in N. benthamiana leaves is used to characterize gene function and has the advantage that up to 15 genes may be co-expressed at the same time to transiently reconstitute entire biosynthetic pathways (Reed et al., 2017; Carqueijeiro et al., 2020). Reconstruction of the artemisinin biosynthetic pathway by transient co-expression of pathway genes (Wallaart et al., 2001; Zhang et al., 2011; Ting et al., 2013; Wang et al., 2016) or stable transformation with pathway genes (Farhi et al., 2011) resulted in the first ectopic production of artemisinin in another plant species (Table 3). Recently, the stable transformation of the moss (P. patens) with artemisinin pathway genes, demonstrated that this compound may also be produced in much more primitive plant species (Ikrum et al., 2017; Table 3 and Figure 6). As a plant-based production platform of artemisinin, moss has been shown to have promiscuous substrate recognition which may be a substitute for some artemisinin biosynthetic pathway genes which are not present in for example N. benthamiana. Substrate promiscuity of sesquiterpenoids pathway from A. annua and Tanacetum parthenium for individual enzymes or pathways is previously reported (Beyragherd Kashkooli et al., 2019). Besides, the simple purification step (due to lack of conjugation phenomenon) has been also stated as one of the advantages of this platform compared to the N. benthamiana.

Artemisinin Production in Yeast

Synthetic biology techniques play an important role in the exploration, overproduction, and structure diversification of phytochemicals (Qi et al., 2015; Muhammad et al., 2020; Alam et al., 2021). The full set of artemisinin biosynthesis genes have also been introduced into yeast, resulting in substantial production of dihydroartemisinic acid in fermenters (Immethun et al., 2013; Paddon et al., 2013; Tang et al., 2014; Table 4). This dihydroartemisinic acid can subsequently photo-chemically be converted to (dihydro)artemisinin (Paddon and Keasling, 2014). Some of the issues that play a role in the potential boosting of artemisinin production in A. annua as discussed above, also play a role in boosting artemisinin production in heterologous hosts. For instance, high activity of HMGR is also important for the ectopic production of artemisinin in yeast (Pitera et al., 2007; Ye and Bhatia, 2012; Tang et al., 2014) (Table 4).

CONCLUSION

Whether artemisinin can be used to not only combat malaria, but also other diseases, including those acting on a pandemic scale, will very much depend on further validation of the efficacy of artemisinin in these other diseases and on how this compound can cost-effectively be made available to the community. Multiple and complementary approaches may be necessary to boost synthesis capacity, varying from the transformation of A. annua itself to boost artemisinin yield, to investment into heterologous production platforms that may be easier to scale up. At the same time, we should not ignore the lessons learned from monotherapy in combating disease, as this may result in the emergence of artemisinin-resistance, as currently happening for malaria. Therefore, both for malaria, as for the potential of artemisinin in combating COVID-19 and other viral infections ACTs or triple artemisinin-based combination therapies (TACTs) may be required to prevent the rise of artemisinin resistant disease variants.

AUTHOR CONTRIBUTIONS

ABK conceptualized the review. KF-K, AR, AB, ARK, and ABK wrote the manuscript. ABK, ARK, and AB reviewed the manuscript. KF-K, AR, and ABK did the figures visualization. All authors contributed to the article and approved the submitted version.

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REFERENCES

Aftab, T., Khan, M. M. A., Idrees, M., Naeem, M., and Moinuddin, (2011). Optimizing nitrogen levels combined with gibberellic acid for enhanced yield, photosynthetic attributes, enzyme activities, and artemisinin content of Artemisia annua. Front. Agric. China 5:51–59. doi: 10.1007/s11703-011-1065-7

Aftab, T., Masroor, M., Khan, A., Idrees, M., Naeem, M., and Moinuddin. (2010). Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisinin production in Artemisia annua L. J. Crop Sci. Biotechnol. 13, 183–188. doi: 10.1007/s12392-010-0040-3

Alam, K., Han, J., Zhang, Y., and Li, A. (2021). Synthetic biology-inspired strategies and tools for engineering of microbial natural product biosynthetic pathways. Biotechnol. Adv. 49:107759. doi: 10.1016/j.biotechadv.2021.107759

Alam, P., and Abdin, M. Z. (2011). Over-expression of HMG-CoA reductase and amorph-4,11-diene synthase genes in Artemisia annua L. and its influence on artemisinin content. Plant Cell Rep. 30, 1919–1928. doi: 10.1007/s00299-011-1099-6

Alcântara, D. D. F. Á, Ribeiro, H. F., Cardoso, P. C., dos, S., Araújo, T. M. T., Burbano, R. R., et al. (2013). In vitro evaluation of the cytotoxic and genotoxic effects of artemether, an antimalarial drug, in a gastric cancer cell line (PG100). J. Appl. Toxicol. 33, 151–156. doi: 10.1002/jat.1734

Aldieri, E., Atragene, D., Bergandi, L., Riganti, C., Costamagna, C., Bosia, A., et al. (2003). Artemisinin inhibits inducible nitric oxide synthase and nuclear factor NF-κB activation. FEBS Lett. 552, 141–144. doi: 10.1016/S0014-5793(03)0095-0

Al-Qurainy, F., and Khan, S. (2010). Mutational approach for enhancement of artemisinin content. Acta Pharmacologica Sinica 31, 1919–1928. doi: 10.1007/s10279-011-0493-2

Anthony, J. R., Anthony, L. C., Nowroozi, F., Kwon, G., Newman, J. D., and Bhatia, 2012; Tang et al., 2014) (Table 4).
endoperoxides involves ROS-dependent depolarization of the membrane potential. *J. Antimicrob. Chemother.* 69, 1005–1016. doi: 10.1093/jac/dkt486
Aguil, S., Husaini, A. M., Abdin, M. Z., and Rather, G. M. (2009). Overexpression of the HMGI-CoA reductase gene leads to enhanced artemisinin biosynthesis in transgenic *Artemisia annua* plants. *Planta Med.* 75, 1453–1458. doi: 10.1055/s-0029-1185775
Krishna, S., Augustin, Y., Wang, J., Xu, C., Staines, H. M., Platteeuw, H., et al. (2021). Repurposing antimarials to tackle the COVID-19 pandemic. *Trends Parasitol.* 37, 8–11. doi: 10.1016/j.pt.2020.10.000
Bahrami, M., Kamalinejad, M., Latifi, S. A., and Seif, F., and Dadmehr, M. (2020). Synergistic antioxidant activity of artesunate and HDAC inhibitors through elevated heve synthesis via synergistic upregulation of ALAS1 expression. *Acta Pharm. Sin. B.* 9, 917–951. doi: 10.1016/j.apsb.2019.05.001
Chen, D. H., Ye, H. C., and Li, G. F. (2000). Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. *Plant Sci.* 155, 179–185. doi: 10.1016/S0168-9452(00)00217-X
Chen, G., Klimkhammer, P. G. L., Escobar-Bravo, R., and Leiss, K. A. (2018). Type VI glanular trichome density and their derived volatiles are differently induced by jasmonic acid in developing and fully developed tomato leaves: implications for thrips resistance. *Plant Sci.* 276, 87–98. doi: 10.1016/j.plantsci.2018.08.007
Chen, H., Sun, B., Pan, S., Jiang, H., and Sun, X. (2009). Dihydroartemisinin inhibits growth of pancreatic cancer cells in vitro and in vivo. *Anticancer. Drugs* 20, 131–140. doi: 10.1074/CAD.08.0132e283212ade
Chen, H., Sun, B., Wang, S., Pan, S., Gao, Y., and Bai, X., et al. (2010). Growth inhibitory effects of dihydroartemisinin on pancreatic cancer cells: involvement of cell cycle arrest and inactivation of nuclear factor-κB. *J. Cancer Res. Clin. Oncol.* 136, 967–903. doi: 10.1007/s00432-009-0731-0
Chen, H. H., Zhou, H. J., and Fang, X. (2003). Inhibition of human cancer cell line growth and human umbilical vein endothelial cell angiogenesis by artemisinin derivatives in vitro. *Pharmacol. Res.* 48, 231–236. doi: 10.1016/S1043-6618(03)00107-5
Chen, K., Shou, L. M., Fu, F., Duan, W. M., Wu, M. Y., Xie, X., et al. (2014). Artesunate induces G2/M cell cycle arrest through autophagy induction in breast cancer cells. *Anticancer. Drugs* 25, 652–662. doi: 10.1074/CAD.0000000000000089
Chen, W., Wang, Y., Lim, T. K., Lin, W. H., Lin, Q., Wang, J., et al. (2017). Artesunate activates the intrinsic apoptosis of HCT116 cells through the suppression of fatty acid synthesis and the NF-κB pathway. *Molecules* 22:1272. doi: 10.3390/molecules22081272
Chen, X., Zhang, X. L., Zhang, G. H., and Gao, Y. F. (2019). Artemisinin promotes Th1 differentiation from CD4+ T cells to enhance cell apoptosis in ovarian cancer via miR-142. *Brazilian J. Med. Biol. Res.* 52, 1–8. doi: 10.1590/1419-431x20197992
Chen, Y., Shen, Q., Wang, Y., Wang, T., Wu, S., Zhang, L., et al. (2013). The stacked-overexpression of FPS, CYP71AV1 and CPR genes leads to the increase of artemisinin concentration and yield in response to optimization of nitrogen and potassium supply to *Artemisia annua* L. *Plant Sci.* 104, 315–323. doi: 10.1093/aob/mcp126
Chen, Y., Feng, Y. H., Gao, L. W., Li, X. Y., Jin, Q. X., Wang, Y. Y., et al. (2019). Artemisinin enhances the anti-tumor immune response in 4T1 breast cancer cells in vitro and in vivo. *Int. Immunopharmacol.* 70, 110–116. doi: 10.1016/j.intimp.2019.01.041
Carqueijeiro, I., Langley, C., Grzech, D., Koudounas, K., Papon, N., O’Connor, S. E., et al. (2020). Beyond the semi-synthetic artemisinin: metabolic engineering of plant-derived anti-cancer drugs. *Curr. Opin. Biotechnol.* 65, 17–24. doi: 10.1016/j.copbio.2019.11.017
Chaijareonkul, W., Viyanant, V., Mahavorasirikul, W., and Na-Bangchang, K. (2011). Cytotoxic activity of artemisinin derivatives against cholangiocarcinoma (CL-6) and hepatocarcinoma (Hep-G2) cell lines. *Asian Pacific J. Cancer Prev.* 12, 55–59.
Charles, D. J., Gebert, E., and Simon, J. E. (1991). Characterization of the essential oil of *Artemisia annua* L. *J. Essent. Oil Res.* 3, 33–39. doi: 10.1080/10412905.1991.9697993
Chen, C., Chen, K., Feng, Z., Wen, X., and Sun, H. (2019). Synergistic antioxidant activity of artesunate and HDAC inhibitors through elevated heve synthesis via synergistic upregulation of ALAS1 expression. *Acta Pharm. Sin. B.* 9, 917–951. doi: 10.1016/j.apsb.2019.05.001
D’Alessandro, S., Scaccabarozzi, D., Signorini, L., Perego, F., Ilboudo, D. P., Ferrante, P., et al. (2020). The use of antimalarial drugs against viral infection. *ACS Infect. Dis.* 6, 2524–2531. doi: 10.1021/acsinfecdis.0c00522
De Oliveira, V. M., da Rocha, M. N., Magalhães, E. P., da Silva Mendes, F. R., Marinho, M. M., de Menezes, R. R. P. P. B., et al. (2021). Computational approach towards the design of artemisinin-thymoquinone hybrids against microorganisms. Microorganisms 8, 1–26. doi: 10.3390/microorganisms8010085
Davies, M. J., Atkinson, C. J., Burns, C., Woolley, J. G., Hippa, N. A., Arroz, R. J. R., et al. (2009). Enhancement of artemisinin concentration and yield in response to optimization of nitrogen and potassium supply to *Artemisia annua*. *Ann. Bot.* 104, 315–323. doi: 10.1093/aob/mcp126
main protease of SARS-COV-2. Futer. J. Pharm. Sci. 7, 1–20. doi: 10.1186/s43094-021-00334-z
Dewick, P. M. (2009). *Medicinal Natural Products: A Biosynthetic Approach*, 3rd Edn. New York, NY: Wiley.

Dietrich, J. A., Yoshikuni, Y., Fisher, K. J., Woolard, F. X., Ockey, D., McPhee, D. J., et al. (2009). A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450BM3. *ACS Chem. Biol.* 4, 261–267. doi: 10.1021/cb900006h

Dilshad, E., Cusido, R. M., Palazon, J., Estrada, K. R., Bonfill, M., and Mirza, B. (2015). Enhanced artemisinin yield by expression of rol genes in Artemisia annua. *Malar. J.* 14, 1–10. doi: 10.1186/s12936-015-0951-5

Dubrow, G. L., Baeg, A. C., Kierpiec, K. A., Yuan, H., Centeno, J. A., Thibodeaux, C. A., et al. (2005). Dihydroartemisinin is cytotoxic to papillomavirus-expressing epithelial cells in vitro and in vivo. *Cancer Res.* 65, 10854–10861. doi: 10.1158/0008-5472.CAN-05-1216

Dong, F., Zhou, X., Li, C., Yan, S., Deng, X., Cao, Z., et al. (2014). Dihydroartemisinin targets VEGFR2 via the NF-κB pathway in endothelial cells to inhibit angiogenesis. *Cancer Biol. Ther.* 15, 1479–1488. doi: 10.4161/15384047.2014.955728

Dong, H. Y., and Wang, Z. F. (2014). Antitumor effects of artemesin on human breast carcinoma MCF-7 cells and IGF-IR expression in nude mice xenografts. *Clin. J. Cancer Res.* 26, 200–207. doi: 10.3978/j.issn.1000-9604.2014.04.07

Dong, L., Hu, S., and Gao, J. (2020). Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov. Ther.* 14, 58–60. doi: 10.1021/acsptsci.0c00222

Edn. New York, NY: Wiley.

Fuzimoto, A. D. (2021). An overview of the anti-SARS-CoV-2 properties of *Artemisia annua*, its antiviral action, protein-associated mechanisms, and repurposing for COVID-19 treatment. *J. Integr. Med.* 19, 375–388. doi: 10.1016/j.jiim.2021.07.003

Frohlich, T., Ndreishkijana, B., Muenzner, J. K., Reiter, C., Hofmeister, E., Mederer, S., et al. (2017). Synthesis of novel hybrids of thymoquinone and artemisinin with hig activity and selectivity against colon cancer. *Chem. Med. Chem.* 12, 2276–234. doi: 10.1002/cmc.21065

Fuentes, P., Zhou, F., Erban, A., Karcher, D., Kopka, J., and Bock, R. (2016). A new synthetic biology approach allows transfer of an entire metabolic pathway from a medicinal plant to a biomass crop. *Elife* 5, 1–26. doi: 10.7554/elife.13664

Gendrot, M., Duflot, J., Boxberger, M., Delandre, O., Jardot, P., De, G. J., Yi, H., Zang, C., Yang, M. Y., et al. (2020). Artemether, artemisinin, and artemisinin-based combination therapies (ACT) and COVID-19 in Africa: in vitro inhibition of SARS-CoV-2 replication by melquine-artesunate. *Int. J. Infect. Dis.* 99, 437–440. doi: 10.1016/j.ijid.2020.08.032

Gong, Y., Gallis, B. M., Goodlett, D. R., Yang, Y., Lu, H., Lacoeste, E., et al. (2013). Effects of transferrin conjugates of artemisinin and artemisinin dimer on breast cancer cell lines. *Anticancer Res.* 33, 123–132.

Graham, L. A., Besser, K., Bluemer, S., Branigan, C. A., Czechowski, T., Elias, L., et al. (2010). The genetic map of *Artemisia annua* identifies loci affecting yield of the antimalarial drug artemisinin. *Science* 327, 328–331. doi: 10.1126/science.1182612

Greenshields, A. L., Shepherd, T. G., and Hoskin, D. W. (2017). Contribution of reactive oxygen species to ovarian cancer cell growth arrest and killing by the anti-malarial drug artesunate. *Mol. Carcinog.* 56, 75–93. doi: 10.1002/mcc.22474

Gu, Y., Wang, X., Wang, X., Yuan, M., Wu, G., Hu, J., et al. (2012). Artemisinin attenuates post-infarct myocardial remodeling by down-regulating the NF-κB pathway. *Tsukuba J. Exp. Med.* 227, 161–170. doi: 10.1620/jem.227.161

Guastalegname, M., and Vallone, A. (2020). Could chloroquine /hydroxychloroquine be harmful in coronavirus disease 2019 (COVID-19) treatment? *Clin. Infect. Dis.* 71, 888–889. doi: 10.1093/cid/ciaa321

Hamacher-Brady, A., Stein, H. A., Turschner, S., Toegel, I., Mora, R., Jennewein, N., et al. (2011). Artemesinates mitochondrial apoptosis in breast cancer cells via iron-catalyzed lysosomal reactive oxygen species production. *J. Biol. Chem.* 286, 6587–6601. doi: 10.1074/jbc.M110.120047

Han, J., Wang, H., Kangarajan, S., Hao, M., Lundgren, A., and Brodelsey, P. E. (2016). Promoting artemisinin biosynthesis in *Artemisia annua* plants by substrate channeling. *Plant Mol. Biol.* 9, 496–498. doi: 10.1007/jmpol.2016.03.004

Hao, D. L., Xie, R., De, G. J., Yi, H., Zang, C., Yang, M. Y., et al. (2020). PH-responsive artemesin polymer prodrugs with enhanced ablation effect on rodent xenograft colon cancer. *Int. J. Nanomedicine* 15, 1771–1786. doi: 10.2147/IJN.S242032

Hassanpour, S., Arab-Zozani, M., Amani, B., Heidarzad, F., Fathalipour, M., and Martinez-de-Hoyo, R. (2021). The efficacy and safety of favipiravir in treatment of COVID-19: a systematic review and meta-analysis of clinical trials. *Sci. Rep.* 11, 1–11. doi: 10.1038/s41598-021-9055-1

Heymann, D. L., and Shindo, N. (2020). COVID-19: what is next for public health? *Lancet* 395, 542–545. doi: 10.1016/S0140-6736(20)30374-3

Hu, W., Chen, S. S., Zhang, J. L., Lou, X. E., and Zhou, H. J. (2014). Dihydroartemisinin induces autophagy by suppressing NF-κB activation. *Cancer Lett.* 343, 239–248. doi: 10.1016/j.canlet.2013.09.035

Hu, Y., Liu, M., Qin, H., Lin, H., An, X., Shi, Z., et al. (2021). Artemesin, artemesin, ebinachin, licochalcone B and andrographolide effectively inhibit SARS-CoV-2 and related viruses in vitro. *Front. Cell Infect. Microbiol.* 11:1–8. doi: 10.3389/fcmi.2021.680127

Huang, J., Tao, G., Liu, J., Cai, J., Huang, Z., and Chen, J. X. (2020). Current status of COVID-19: natural products and herbal medicine. *Front. Pharmacol.* 11:1–18. doi: 10.3389/fphar.2020.588508

Hwang, Y. P., Yun, H. J., Kim, H. G., Han, E. H., Lee, G. W., and Jeong, H. G. (2010). Suppression of PMA-induced tumor cell invasion by dihydroartemisinin via
inhibition of PKCa/Raf/MAPKs and NF-κB/AP-1-dependent mechanisms. *Biochem. Pharmacol.* 79, 1714–1726. doi: 10.1016/j.bpc.2010.02.003 Iannicelli, J., Guariniello, J., Tossi, V. E., Regalado, J. J., Di Ciaccio, L., van Baren, C. M., et al. (2020). The "polyeploid effect" in the breeding of aromatic and medicinal species. *Sci. Hort.* 260:108854. doi: 10.1016/j.scienta.2019.108854 Ikram, N. K. B., Beyraghdar Kashkooli, A., Peramuna, A. V., van der Krol, A. R., Bouwmeester, H., and Simonsen, H. T. (2019). Insights into heterologous biosynthesis of arteannuin B and artemisinin in phycocriptrella patens. *Molecules* 24:822. doi: 10.3390/molecules24123822 Ikram, N. K. B. K., Beyraghdar Kashkooli, A., Peramuna, A. V., van der Krol, A. R., Bouwmeester, H., and Simonsen, H. T. (2017). Stable production of the antimalarial drug artemisinin in the moss phycocriptrella patens. *Front. Bioeng. Biotechnol.* 5:1–8. doi: 10.3389/fbioe.2017.00047 Ikram, N. K. B. K., and Simonsen, H. T. (2017). A review of biotechnological artemisinin production in plants. *Front. Plant Sci.* 8:1–10. doi: 10.3389/fpls.2017.01966 Ilamathi, M., Prabu, P. C., Ayyappa, K. A., and Sivaramakrishnan, V. (2016). Artesunate differentially regulates expression of myogenic regulatory factors in muscle satellite cells. *J. Endocrinol.* 230, 137–147. doi: 10.1677/JOE-15-0339 Ilimani, L., Satter, L., and Kapepula, P. (2015). Artemisinins in Combating Different Diseases. *Frontiers in Plant Science* | www.frontiersin.org 17 February 2022 | Volume 13 | Article 780257
Liao, P., Hemmerlin, A., Bach, T. J., and Chye, M. (2016). The potential of Artemisia annua in combating different diseases. Frontiers in Plant Science | www.frontiersin.org 18 10.1038/celldisc.2017.42

Farmanpour-Kalalagh et al. Artemisinins in Combating Different Diseases

Li, W., Ma, G., Deng, Y., Wu, Q., Wang, Z., and Zhou, Q. (2021). Artesunate exhibits synergistic anti-cancer effects with cisplatin on lung cancer A549 cells by inhibiting MAPK pathway. Gene 766:145314. doi: 10.1016/j.gene.2020.145314

Li, X., Ba, Q., Liu, Y., Yue, Q., Chen, P., Li, J., et al. (2017). Dihydroartemisinins selectively inhibits PDGFRα-positive ovarian cancer growth and metastasis through inducing degradation of PDGFRα protein. Cell. Discov. 3, 1–13. doi: 10.1038/celldisc.2017.42

Li, X., Gu, S., Sun, D., Dai, H., Chen, H., and Zhang, Z. (2018). The selectivity of artemisinin-based drugs on human normal and cancer cells. Environ. Toxicol. Pharmacol. 57, 86–94. doi: 10.1016/j.etap.2017.12.004

Li, Y., Sui, H., Jiang, C., Li, S., Han, Y., Huang, P., et al. (2018). Dihydroartemisinin increases the sensitivity of photodynamic therapy via NF-κB/HIF-1α/VEGF pathway in esophageal cancer cell in vitro and in vivo. Cell Physiol. Biochem. 48, 2035–2045. doi: 10.1007/s00299-018-5245-1

Li, Y. J., Zhou, J. H., Du, X. X., Jia, D. X., Wu, C. L., Huang, P., et al. (2014). Dihydroartemisinin accentuates the anti-tumor effects of photodynamic therapy via inactivation of NF-κB in Eca109 and EC9706 esophageal cancer cells. Cell Physiol. Biochem. 33, 1527–1536. doi: 10.1007/s00587-016-4887-3

Liang, W., Liu, J., Wu, H., Qiao, X., Xu, X., Liu, Y., et al. (2019). Artemisinin induces reversal of EMT affects the molecular biological activity of ovarian cancer SKOV3 cell lines. Oncol. Lett. 18, 3407–3414. doi: 10.3892/ol.2019.10608

Liao, P., Hemmerlin, A., Bach, T. J., and Chye, M. (2016). The potential of the cytosol leads to high arteannuin B production and artemisinin increase. J. Nat. Prod. 91, 466–479. doi: 10.1039/c5np00978c

Ma, D., Pu, G., Lei, C., Ma, L., Wang, H., Guo, Y., et al. (2009). Isolation and characterization of AaWRKY1, an artemisinin induction transcription factor that regulates the amorph-4,11-diene synthase gene, a key gene of artemisinin biosynthesis. Plant Cell Physiol. 50, 2146–2161. doi: 10.1093/pcp/pcp1149

Ma, D. M., Wang, Z., Wang, L., Alejos-Gonzales, F., Sun, M. A., and Xie, D. Y. (2015). A genome-wide scenario of terpenic pathways in self-pollinated Artemisia annua. Mol. Plant 8, 1580–1598. doi: 10.1093/mp/amu107

Ma, H., Yao, Q., Zhang, A. M., Lin, S., Wang, X. X., Wu, L., et al. (2011). The effects of artemisinin on the expression of EGFR and ABCG2 in A549 human lung cancer cells and a xenograft model. Molecules 16, 10356–10369. doi: 10.3390/molecules161210556

Ma, Y.-N., Xu, D.-B., Li, L., Zhang, F., Fu, X.-Q., Shen, Q., et al. (2018). Jasmonate synergistically promotes artemisinin biosynthesis by activating the TCPI4-ORA complex in Artemisia annua. Sci. Adv. 4:eaaas9357. doi: 10.1126/sciadv.aas9357

Malhotra, K., Subramanian, M., Rawat, K., Kalamuddin, M., Qureshi, M. I., Malhotra, P., et al. (2016). Compartmentalized metabolic engineering for artemisinin biosynthesis and effective malaria treatment by oral delivery of plant cells. Mol. Plant 9, 1464–1477. doi: 10.1093/mp/ MPL019

Mandal, S., Upadhyay, S., Wajid, S., Ram, M., Jain, D. C., Singh, V. P., et al. (2014). Arbuscular mycorrhiza increases artemisinin accumulation in Artemisia annua by high expression of key biosynthesis genes via enhanced jasmonic acid levels. Mycorrhiza 25, 1356–1369. doi: 10.1007/s00572-014-0614-3

Mani, J. S., Johnson, J. B., Steel, J. C., Brossczak, D. A., Neilsen, P. M., Walsh, K. B., et al. (2020). Natural product-derived phytochemicals as potential agents against coronaviruses: a review. Virus Res. 284:197989. doi: 10.1016/j.virusres.2020.197989

Mao, H., Gu, H., Qu, X., Sun, J., Song, B., Gao, W., et al. (2013). Involvement of the mitochondrial pathway and Bim/Bcl-2 balance in dihydroartemisinin-induced apoptosis in human breast cancer in vitro. Int. J. Mol. Med. 31, 213–218. doi: 10.3892/ijmm.2012.1176

Martin, V. J. J., Pitera, D. J., Withers, S. T., Newman, J. D., and Keasling, J. D. (2008). Natural product-derived phytochemicals as potential agents against coronaviruses: a review. Virus Res. 284:197989. doi: 10.1016/j.virusres.2020.197989

Mengue, A., Bhattacharjee, S., Pandharkar, T., Liu, H., Estiu, G., Stahelin, R. V., et al. (2010). A microbial model for dihydroartemisinin production. Int. J. Mol. Med. 25, 863–867. doi: 10.3892/ijmm.2010.361

Merdka, P., Mercke, P., Bengtsson, M., Bouwmeester, H. J., Posthumus, M. A., and Brodelius, P. E. (2000). Molecular cloning, expression, and characterization of amorpha-4,11-diene synthase, a key enzyme of artemisinin biosynthesis in Artemisia annua. J. Biol. Chem. 275, 180–184. doi: 10.1074/jbc.M004024200

Poole, K. J., et al. (2000). Artemisinins in Combating Different Diseases

Poole, K. J., et al. (2000). Artemisinins in Combating Different Diseases

Poole, K. J., et al. (2000). Artemisinins in Combating Different Diseases
Mok, S., Ashley, E. A., Ferreira, P. E., Zhu, L., Lin, Z., Yeo, T., et al. (2015). Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science 347, 431–435. doi: 10.1126/science.1260403

Mondal, A., and Chatterjee, U. (2015). Artemisinin represses telomerase subunits and induces apoptosis in HPV-19 infected human cervical cancer cells. J. Cell. Biochem. 116, 1968–1981. doi: 10.1002/jcb.25152

Muhammad, A., Feng, X., Rasool, A., Sun, W., and Li, C. (2020). Production of Artemisia annua L. hot-water extracts show potent activity in vitro against Covid-19 variants including delta. J. Ethnopharmacol. 284, 114779. doi: 10.1016/j.jep.2021.114779

Nair, M. S., Huang, Y., Fidock, D. A., Towler, M. J., and Weathers, P. J. (2022). Evaluation of Artemisia annua L. as a potential source of dihydroartemisinin. Phyther. Res. 35, 1329–1344. doi: 10.1002/ptr.6895

Newman, J. D., Marshall, J., Chang, M., Nowroooz, F., Paradise, E., Pitera, D., et al. (2006). High-level production of amorpha-4,11-diene in a two-phase partitioning bioreactor of metabolically engineered Escherichia coli. Biotechnol. Bioeng. 95, 684–691. doi: 10.1002/bit.20117

Nie, C., Trimpert, J., Moon, S., Haag, R., Gilmore, K., Kaufer, B. B., et al. (2021). In vitro efficacy of artemisia extracts against SARS-CoV-2. Viral. J. 18, 1–7. doi: 10.1128/ijsv.01285-02-01651-8

Nunes, J. J., Pandey, S. K., Yadav, A., Goel, S., and Ateeq, B. (2017). Targeting NF-kappa B signalling by artemisinate restores sensitivity of castrate-resistant prostate cancer cells to antiandrogens. Neoplasia 19, 333–345. doi: 10.1016/j.neo.2017.02.002

Ohgami, Y., Elstad, C. A., Chung, E., Shirachi, D. Y., Quock, R. M., and Lai, H. C. (2010). Effect of hyperbaric oxygen on the anticancer effect of artemisinin on molt-4 human leukemia cells. Anticancer Res. 30, 4467–4470.

O’Neill, P. M., Barton, V. E., and Ward, S. A. (2010). The molecular mechanism of action of artemisinin-the debate continues. Molecules 15, 1705–1732. doi: 10.3390/molecules15031705

Ooko, E., Saeed, M. E. M., Ribaudo, G., Coghi, P., Yang, L. J., Ng, J. P. L., Masri, A. M., and Memos, M. (2021). Artemisia annua L. through induced mutation. J. Cell. Physiol. 236, 1054–1066. doi: 10.1002/jcp.29363

Parshukov, I. A., Netrusov, A. L. and Sutherland, J. B. (2012). Microbial transformation of antimalarial terpenoids. Biotechnol. Adv. 30, 1516–1523. doi: 10.1016/j.biotechadv.2012.03.010

Pandey, N., and Pandey-Rai, S. (2014). Short term UV-B radiation-mediated transcriptional responses and altered secondary metabolism of in vitro propagated plantlets of Artemisia annua L. Plant Cell, Tissue Organ Cult. 116, 371–385. doi: 10.1007/s11240-013-0413-0

Paradise, E. M., Kirby, J., Chan, R., and Keasling, J. D. (2008). Redirection of flux through the FPP branch-point in Saccharomyces cerevisiae by down-regulating squalene synthase. Biotechnol. Bioeng. 100, 371–378. doi: 10.1002/bit.21576

Paddon, C. J., Westfall, P. J., Pitera, D. J., Benjamin, K., Fisher, K., McPhee, D., et al. (2013). High-level semi-synthetic production of the potent antimalarial artemisinic acid in engineered yeast. Metab. Eng. 22, 1045–1054. doi: 10.1016/j.ymben.2015.08.002

Pahl, H. L. (1999). Activators and target genes of Rel/NF-kB transcription factors. Oncogene 18, 6853–6866. doi: 10.1038/sj.onc.1203239

Raymond, M., Miriam, K., Oliver, K., Edwin, M., and Stephen, K. (2015). Artemisinin biosynthesis in growing plants of Artemisia annua through induced mutation. Curr. Chem. Biol. 7, 1–13. doi: 10.1007/s11240-014-0434-2

Ribaudo, G., Coghi, P., Yang, L. J., Ng, J. P. L., Masri, A. M., and Memos, M. (2021). Computational and experimental insights on the interaction of artemisinin, dihydroartemisinin and chloroquine with SARS-CoV-2 spike protein-receptor binding-domain (RBD). New. J. Biol. 33, 1–6. doi: 10.1007/s14786419.2021.1925894

Riganti, C., Doublier, S., Viarisio, D., Miraglia, E., Ghigo, D., et al. (2009). Artemisinin induces doxorubicin resistance in human colon cancer cells via calcium-dependent activation of HIF-1α and p-glycoprotein overexpression. Br. J. Pharmac. 156, 1054–1066. doi: 10.1111/j.1476-5381.2009.01117.x

Ro, D. K., Ouettel, M., Paradise, E. M., Burd, H., Eng, D., Paddon, C. J., et al. (2008). Induction of multiple pleiotropic drug resistance genes in yeast engineered to produce an increased level of anti-malarial drug precursor, artemisinic acid. BMC Biotechnol. 8, 14–14. doi: 10.1186/1472-6750-8-83

Ro, D. K., Paradise, E. M., Quellet, M., Fisher, K. J., Newman, K. L., Ndungu, J. M., et al. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440, 940–943. doi: 10.1038/nature04640

Roh, J. L., Kim, E. H., Jang, H., and Shin, D. (2017). Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. Redox Biol. 11, 254–262. doi: 10.1016/j.redox.2016.12.010

Rolta, R., Salaria, D., Kumar, V., Sourirajan, A., and Dev, K. (2000). Phytoconstituents of Rheum emodi, Thymus serphyllum and Artemisia annua inhibit COVID-19 binding to ACE2 receptor: in silico approach. Curr. Pharmacol. Rep. doi: 10.21203/rs.3.rs-30938v1 [PubMed ahead of print],

Sa, G., Mi, M., He-chun, Y., Ben-ye, L., Guo-feng, L., and Kang, C. (2001). Effects of ipt gene expression on the physiological and chemical characteristics of Artemisia annua L. Plant Sci. 160, 691–698. doi: 10.1016/S0168-9450(00)00453-2

Sarma, B., Willmes, C., Angerer, L., Adam, C., Becker, J. C., Kervarrec, T., et al. (2020). Artesunate affects T antigen expression and survival of virus-positive merkel cell carcinoma. Cancers 12, 1–15. doi: 10.3390/cancers12040919

Schramek, N., Wang, H., Römisch-Margl, W., Kell, B., Radzywick, T., Winzenhörlein, B., et al. (2010). Artemisinin biosynthesis in growing plants of

Artemisinins in Combating Different Diseases
Artemisia annua. a 13C/2 study. Phytochemistry 71, 179–187, doi: 10.1016/j.phytochem.2009.10.015

Sehaila, M., and Chemat, S. (2020). Antimalarial-actor artemisinin and derivatives portray more potent binding to Lys333 and Lys31-binding hotspots of SARS-CoV-2 spike protein than hydroxychloroquine: potential repurposing of artemisinol for COVID-19. J. Biomed. Struct. Dyn. 39, 6184–6194. doi: 10.1080/07391102.2020.1796809

Shandilya, A., Chacko, S., Jayaram, B., and Ghosh, I. (2013). A plausible mechanism for the antimalarial activity of artemisinin: a computational approach. Sci. Rep. 3, 1–7. doi: 10.1038/srep02513

Shen, Q., Chen, Y. F., Wang, T., Wu, S. Y., Lu, X., Zhang, L., et al. (2012). Overexpression of the cytochrome P450 monoxygenase (cyp71av1) and cytochrome P450 reductase (cpr) genes increased artemisinin content in Artemisia annua (asteraceae). Genet. Mol. Res. 11, 3298–3309. doi: 10.4293/2012.September.12.13

Shen, Q., Yan, T., Fu, X., and Tang, K. (2016). Transcriptional regulation of artemisinin biosynthesis in Artemisia annua L. Sci. Bull. 61, 18–20. doi: 10.1007/s11434-015-0983-9

Shi, R., Cui, H., Bi, Y., Huang, X., Song, B., Cheng, C., et al. (2015). Artesunate altered cellular mechanical properties leading to deregulation of cell proliferation and migration in esophageal squamous cell carcinoma. Oncol. Lett. 9, 2249–2255, doi: 10.3892/ol.2015.2982

Singh, N. P., and Lai, H. C. (2004). Artemisinin induces apoptosis in human cancer cells. Anticancer Res. 24, 2277–2280.

Singh, N. P., and Verma, K. B. (2002). Case report of a laryngeal squamous cell carcinoma treated with artesunate. Arch. Oncol. 10, 279–280. doi: 10.2298/AOO0204279S

Singhal, T. (2020). A review of coronavirus disease-2019 (COVID-19). Int. J. Oncol. 57, 281–286. doi: 10.3892/ijo.2020.114302

Srivastava, N. K., and Sharma, S. (1990). Influence of micronutrient imbalance on growth and artemisinin content in Artemisia annua. Indian J. Pharm. Sci. 52, 225–227.

Su, T., Li, F., Guan, J., Liu, L., Huang, P., Wang, Y., et al. (2019). Artemisinin action and resistance in Plasmodium falciparum type and relative gene dosage. New. Phyto. 199, 352–366. doi: 10.1111/nphy.12274

Ting, H. M., Wang, B., Rydén, A. M., Woottiez, L., Van Herpen, T., Verstappen, F. W. A., et al. (2013). The metabolite chemotype of Nicotiana benthamiana transiently expressing artemisinin biosynthetic pathway genes is a function of CYP71AV1 type and relative gene dosage. New. Phyto. 199, 352–366. doi: 10.1111/nphy.12274

Tran, K. Q., Tin, A. S., and Firestone, G. L. (2014). Artemisinin triggers a G1 cell cycle arrest of human Ishikawa endometrial cancer cells and inhibits cyclin-dependent kinase-4 promoter activity and expression by disrupting nuclear factor-xB transcriptional signaling. Anticancer. Drugs 25, 270–281. doi: 10.1097/PLA.0000000000000534

Tran, T. H., Nguyen, A. N., Kim, J. O., Yong, C. S., and Nguyen, C. N. (2016). Enhancing activity of artesunate against breast cancer cells via induced-apoptosis pathway by loading into lipid carriers. Artif. Cells Nanomed. Biotechnol. 44, 1979–1987. doi: 10.1007/s10528-015.1129616

Tran, K. Q., Tin, A. S., and Firestone, G. L. (2014). Artemisinin triggers a G1 cell cycle arrest of human Ishikawa endometrial cancer cells and inhibits cyclin-dependent kinase-4 promoter activity and expression by disrupting nuclear factor-xB transcriptional signaling. Anticancer. Drugs 25, 270–281. doi: 10.1097/PLA.0000000000000534

Tsong, Y., Liu, Y., Zheng, H., Zheng, L., Liu, W., Wu, J., et al. (2016). Artemisinin and its derivatives can significantly inhibit lung tumorigenesis and tumor metastasis through Wnt/b-catenin signaling. Oncotarget 7, 31413–31428. doi: 10.18632/oncotarget.8920

Verma, S., Das, P., and Kumar, V. L. (2017). Chemoprevention by artesunate in colorectal cancer treated with artesunate. Anticancer Res. 37, 31–37. doi: 10.21875/37.1.31

Van Herpen, T. W. J. M., Cankar, K., Nogueira, M., Bosch, D., Bouwmeester, H. J., and Beekwilder, J. (2010). Nicotiana benthamiana as a production platform for artemisinin precursors. PLoS One 5:14222. doi: 10.1371/journal.pone.0014222

Vatsveen, T. K., Myhre, M. R., Steen, C. B., Walchli, S., Lingjaerde, O. C., Bai, B., et al. (2018). Artesunate shows potent anti-tumor activity in B-cell lymphoma. J. Hematol. Oncol. 11, 1–12. doi: 10.1186/s12095-018-0561-0

Velazquez-Salinas, L., Verdugo-Rodriguez, A., Rodriguez, L. L., and Borca, M. V. (2019). The role of interleukin 6 during viral infections. Front. Microbiol. 10:6–1. doi: 10.3389/fmicb.2019.01057

Verma, I. M., Stevenson, J. K., Schwarz, E. M., and Van Antwerp, D. (1995). Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. Genes Dev. 9, 2723–2735.

Verma, S., Das, P., and Kumar, V. L. (2017). Chemoprevention by artesunate in a preclinical model of colorectal cancer down regulates NF-kB, suppression of angiogenesis, cellular proliferation and induction of apoptosis. Chem. Biol. Interact. 278, 84–91. doi: 10.1016/j.cbii.2017.10.017

Verma, S., Twilley, D., Esnare, M., Oosthuizen, C. B., Reid, A. M., Nel, M., et al. (2020). Anti-SARS-CoV-1 natural products with the potential to inhibit SARS-CoV-2 (COVID-19). Front. Pharmacol. 11:1–20. doi: 10.3389/fphar.2020.561334

Werpoorte, R., and Memelink, J. (2002). Engineering secondary metabolite biosynthesis in Nicotiana tabacum L. (asteraceae) trichome-specific cDNAs reveal CYP71AV1 type and relative gene dosage. Transient expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin. Plant Cell Rep. 21, 460–465. doi: 10.1007/s002990020336-8

Wang, B., Beyraghdar Kashkooli, A., Sallets, A., Ting, H. M., de Ruijter, N. C. A., Olofsson, L., et al. (2016). Transient production of artemisinin in Nicotiana benthamiana.
Zheng, J., Sun, X., Wang, L., Wong, Y. K., Lee, Y. M., Zhou, C., et al. (2018). Artesunate-induced mitophagy alters cellular redox status. Redox Biol. 19, 263–273. doi: 10.1016/j.redox.2018.07.025

Zheng, L., Jing, F., Li, F., Li, M., Wang, Y., Wang, G., et al. (2009). Development of transgenic Artemisia annua (chinese wormwood) plants with an enhanced content of artemisinin, an effective anti-malarial drug, by hairpin-RNA-mediated gene silencing. Biotechnol. Appl. Biochem. 52:199. doi: 10.1042/BA20080068

Zhang, L. X., Liu, Z. N., Ye, J., Sha, M., Qian, H., Bu, X. H., et al. (2014). Artesunate exerts an anti-immunosuppressive effect on cervical cancer by inhibiting PGF2 production and Foxp3 expression. Cell Biol. Int. 38, 639–646.

Zhang, P., Luo, H. S., Li, M., and Tan, S. Y. (2015). Artesunate inhibits the growth of human gastric cancer cells by downregulating COX-2. *Onco. Targets. Ther.* 8, 845–854. doi: 10.2147/OTT.S81041

Zhang, Y., Nowak, G., Reed, D. W., and Covello, P. S. (2011). The production of artemisinin precursors in tobacco. Plant Biotechnol. J. 9, 445–454. doi: 10.1111/j.1467-7652.2010.00556.x

Zhang, Y., Teoh, K. H., Reed, D. W., Maes, L., Goossens, A., Olson, D. J. H., et al. (2005). Artemisinins in Combating Different Diseases. *Frontiers in Plant Science* | www.frontiersin.org 22

Zhou, M., and Memelink, J. (2016). Jasmonate-responsive transcription factors regulating plant secondary metabolism. *Biotechnol. Adv.* 34, 441–449. doi: 10.1016/j.biotechnadv.2016.02.004

Zhou, X., Chen, Y., Wang, F., Wu, H., Zhang, Y., Liu, J., et al. (2020a). Artesunate induces autophagy dependent apoptosis through upregulating ROS and activating AMPK-mTOR-ULK1 axis in human bladder cancer cells. *Chem. Biol. Interact.* 331:109273. doi: 10.1016/j.cbi.2020.109273

Zhoud, X., Sun, W. J., Wang, W. M., Chen, K., Zheng, J. H., Lu, M. D., et al. (2013). Artesunate inhibits the growth of gastric cancer cells through the mechanism of promoting oncosis both in vitro and in vivo. *Anticancer. Drugs* 24, 920–927. doi: 10.1097/CAD.0b013e328364a109

Zhou, X., Zijistra, S. N., Soto-Gamez, A., Setroikromo, R., and Quax, W. J. (2020b). Artemisinin derivatives stimulate DRS-specific TRAIL-induced apoptosis by regulating wildtype P53. *Cancers (Basel)*. 12:2514. doi: 10.3390/cancers12092514

Zhou, Y., Gilmore, K., Ramirez, S., Settels, E., Gammeltoft, K. A., Pham, L. V., et al. (2021). In vitro efficacy of artesinin-based treatments against SARS-CoV-2. *Sci. Rep.* 11, 1–14. doi: 10.1038/s41598-021-93361-y

Zhou, Y., Wang, X., Zhang, J., He, A., Wang, Y. L., Han, K., et al. (2017). Artesunate suppresses the viability and mobility of prostate cancer cells through UCA1, the sponge of miR-184. *Oncotarget* 8, 18260–18270. doi: 10.18632/oncogarget.15335

Zhu, S., Liu, W., Ke, X., Li, J., Hu, R., Cui, H., et al. (2014). Artemisinin reduces cell proliferation and induces apoptosis in neuroblastoma. *Oncol. Rep.* 32, 1094–1100. doi: 10.3892/or.2014.3323

Zhu, Z., Cai, T., Fan, L., Lou, K., Hua, X., Huang, Z., et al. (2020). Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. *Int. J. Infect. Dis.* 95, 332–339. doi: 10.1016/j.ijid.2020.04.041

Zuo, W., Wang, Z. Z., and Xue, J. (2014). Artesunate induces apoptosis of bladder cancer cells by miR-16 regulation of COX-2 expression. *Int. J. Mol. Sci.* 15, 14298–14312. doi: 10.3390/ijms150814298

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