Targeting autophagy with natural products to prevent SARS-CoV-2 infection

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ARTICLE INFO

Article history:
Received 30 July 2021
Received in revised form 27 September 2021
Accepted 12 October 2021
Available online 14 October 2021

Keywords:
COVID-19
Viropohagy
Coronavirus
Chloroquine
Pandemic
Phytotherapy
Resveratrol

ABSTRACT

Autophagy is a catabolic process that maintains internal homeostasis and energy balance through the lysosomal degradation of redundant or damaged cellular components. During virus infection, autophagy is triggered both in parenchymal and in immune cells with different finalistic objectives: in parenchymal cells, the goal is to destroy the virion particle while in macrophages and dendritic cells the goal is to expose virion-derived fragments for priming the lymphocytes and initiate the immune response. However, some viruses have developed a strategy to subvert the autophagy machinery to escape the destructive destiny and instead exploit it for virion assembly and exocytosis. Coronaviruses (like SARS-CoV-2) possess such ability. The autophagy process requires a set of proteins that constitute the core machinery and is controlled by several signaling pathways. Here, we report on natural products capable of interfering with SARS-CoV-2 cellular infection and replication through their action on autophagy. The present study provides support to the use of such natural products as adjuvant therapeutics for the management of COVID-19 pandemic to prevent the virus infection and replication, and so mitigating the progression of the disease.

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1. Introduction

In December 2019, rose in Wuhan (Hubei Province, China) what would soon become one of the most contagious viral pandemics in recent human history.1 The disease was named Corona Virus Disease 2019 (COVID-19), after the family name of the causative virus SARS-CoV-2. The worse clinical complication of COVID-19 is dominated by a respiratory distress syndrome (Severe Acute Respiratory Syndrome, SARS), which may cause death if untreated. As of 29 July 2021, there are 197 million confirmed cases of COVID-19, and more than 4 million confirmed deaths worldwide (WHO report at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports). Although vaccination constitutes an important and useful means for preventing the spread of the disease, it remains urgent to identify effective strategies to treat at the earliest stages those who get infected. A variety of protocols for treating COVID-19 patients have been proposed, which include antiviral drugs, monoclonal antibodies, antibiotics, and anti-inflammatory drugs in combination and sequential administration depending on the disease stage and the clinical condition of the patient. Natural products provide a source of bioactive molecules that can be exploited for novel and effective treatments to prevent the fatal evolution of this disease.2–6 These natural bio-molecules could interfere at any stage of the virus life cycle, from entering the cell to its replication, assembly, and exit from the cell, as well as by triggering virus clearance.7–10 Autophagy, a vesicular-driven degradation pathway of cellular components, is triggered as a cellular stress response to viral infection, and it is involved in all steps of CoV replication and propagation.11,12

In this review, we will focus on those natural products that have been shown effective in preventing and limiting the infection and replication of CoV through the modulation of the autophagy process. First, we will introduce the principal cellular and molecular features of the autophagy process, then we will discuss how SARS-CoV-2 viral replication interacts with the autophagy-lysosomal features of the autophagy process, then we will discuss how SARS-CoV-2 viral replication interacts with the autophagy-lysosomal...
vesicular traffic, and finally, we will present and discuss the mechanisms of action of the natural products that potentially interfere with these processes.

2. Autophagy at glance

Autophagy (herein referring to macroautophagy) is a catabolic process that maintains cell homeostasis and preserves cell viability under pathological stresses, including viral infections.\(^{11,13}\) The two other known autophagy pathways, namely microautophagy and chaperone-mediated autophagy, play a little if any role in virus infections. Here, we will provide a glance at the autophagy machinery, as a comprehensive description of this process can be found elsewhere.\(^{14,15}\)

Autophagy starts with the formation of a double-membrane vesicle, named the autophagosome that sequesters the substrates to be delivered to the lysosome for full degradation. The core machinery includes more than 30 autophagy-related (ATG) proteins. The main steps and ATG proteins involved in the autophagy process are depicted in Fig. 1. In brief, the autophagosome starts from an ‘omega-shaped’ membrane (the phagophore) in the proximity of the endoplasmic reticulum-Golgi area. Two important events mark this step. First, the cytosolic protein LC3 normally associated with the microtubules (MAP-LC3) is sequentially processed by ATG4 to give two forms of LC3, namely LC3-I and LC3-II. Second, the autophagosome is constructed, the autophagosome substrate (e.g., protein aggregates) to be degraded is bound by the p62/SQSTM1 protein and sequestered into the lumen. In the case of mitophagy, oxidized mitochondria are sequestered via interaction with BNIP3. The autophagosome will eventually fuse with endosomes and lysosomes to form the autolysosome, where the substrates will be released in the cytoplasm for recycling.

The autophagy process is finely tuned by redundant pathways that sense the lack of nutrients and growth factors or energy sources as well as the presence of bacteria and viruses in the cytoplasm or endocytic organelles.\(^{16}\) The main signaling pathways that control autophagy involve the PI3KC1-AKT-mTORC1 negative axis and the AMPK-mTORC1-ULK1 positive axis. The mTORC1 complex is the central hub receiving signals from amino acids, growth factors, and glucose, and controls negatively autophagy by inhibiting the ULK1 complex, whereas AMPK, triggered by the rise of AMP following ATP production impairment (as it occurs, for instance, when glucose is lacking), inhibits mTORC1 and activates ULK1. Downstream to ULK1 is the Vps15-BECLIN1-PI3KC3-ULKC1 complex, known as the “autophagy interactome”, which starts the signal for the autophagosome formation.

The autophagy pathway described above is known as the “canonical” pathway, which is typically active at a basal rate in all eucaryotic cells for keeping the macromolecular homeostasis, and that is hyper-induced under starvation or proteotoxic stress. Besides, other noncanonical pathways have been described where certain ATG proteins (e.g., BECLIN1, ATG5, ATG7) are unnecessary for autophagosome formation.\(^{17,18}\) Alternative non-canonical regulatory pathways apart from the classic mTORC1–ULK1 circuit have also been described.\(^{19}\) For instance, it has been reported the regulation of BECLIN1-dependent autophagy via a MEF/ERK pathway\(^{20}\) and a MAPK/JNK pathway.\(^{21}\) Both canonical and noncanonical pathways are regulated at genetic and epigenetic levels, and thus the two pathways are dynamically modulated and can overlap depending on the metabolic state of the cell and the type of stimulus. To be noted, non-canonical autophagy pathways play a pivotal role in xenophagy, i.e. the lysosomal-mediated destruction of phagocytosed or intracellular localized microbes.\(^{22,24}\) For instance, one such non-canonical autophagy pathway is the so-called Lc3-associated phagocytosis (LAP), where pathogens recognized by Toll-Like Receptors (TLR) are engulfed in single membrane vacuoles (endosomes, phagosomes) decorated with lipidated LC3. Recently, it has been shown that LAP protects from Influenza A Virus (IAV) pathogenicity, limiting viral replication in the lungs and preventing lung inflammation.\(^{25}\) Another non-canonical autophagy pathway is the so-called secretory autophagy, which can be hijacked by viruses for their engulfment into exosomes from where they are released and thus spread to other cells.\(^{24}\)
3. Mechanisms of SARS-CoV-2 cell infection and replication: interaction with the autophagy-lysosome system

Coronaviruses (CoVs) genome is made up of a long (~30 kb) single-stranded positive-sense RNA, typically organized in a 5’ cap structure, followed by the leader sequence, the untranslated region (UTR), the sequences coding for replicase polyproteins, structural and accessory proteins, and finally the 3’ UTR along with a polyA tail.26 The SARS-CoV-2 genome includes at the 5’-terminal two overlapping open reading frames (ORF1a and 1b) encoding two polyproteins (pp1a/1ab) that eventually are processed into 16 non-structural proteins (nsps), followed at the 3’-terminal by the coding sequences for the main structural proteins S (Spike), E (Envelope), M (Membrane) and N (Nucleocapside). Moreover, many other accessory proteins are involved in pp1a/pp1b processing, such as the chymotrypsin-like protease (3CL\textsuperscript{PRO}; aka Main protease M\textsuperscript{PRO}) and papain-like proteinases (PL\textsubscript{1}PRO and PL\textsubscript{2}PRO) and in genome replication\(^2\) (see also Fig. 3 below). Nsps, arising from the processing of pp1a/1 ab, are necessary to form the replication-transcription complex (RTC). The S protein (~180 kDa), a class I fusion protein, forms homotrimers that mediate the attachment to human angiotensin-converting enzyme 2 (hACE2) on the host cell surface through its receptor-binding domain (RBD). The M protein (25–30 kDa) is crucial for promoting both the induction of membrane curvature and the binding to the N protein, while E protein is necessary for assembly and release of the viral genome. Lastly, N protein interacts with M protein and nsps3, a component of RTC, to promote the packaging of the viral genome into the viral particles.28

The mechanisms through which CoV enter the cells, replicate within, and exit from them are illustrated in Fig. 2. In brief, CoV life cycle begins once the virus has entered the target host cell. The main path for CoV entry is via clathrin-mediated or clathrin/caveolae-independent endocytosis.\(^8,20\) The endocytosed virus can be delivered to the autophagy-lysosomal organelles for degradation. Within the endosome, the β-CoV ssRNA triggers the Toll-like receptor (specifically TLR7 and TLR8) response.\(^30\) However, the virus RNA can escape from endocytic vesicles (upon cathepsin L-mediated processing of S and virus envelope-membrane fusion), and relocate in the cytoplasm, where here again it could (or not) be trapped within autophagosomes (which also are provided with TLRs). Additionally, the virus may enter the cell by lipid blending of the virus envelope and the host cell membrane. Cells expressing ACE2 on the membrane are specifically targeted by SARS-CoV-2 through the S protein.\(^31\) Whichever the path used for entering the cell, the cleavage of the S protein into the subunits S1 and S2 by host proteases such as endosomal cathepsin L, furin, trypsin, transmembrane protease serine protease 2 (TMPRSS-2), or human airway trypsin-like protease is an obligatory step for allowing the fusion between the viral envelope and host cell membranes (either endosomal or plasma membrane) and the release of the genome into the cytoplasm.\(^31,32\) Accordingly, inhibition of this proteolytic step greatly reduces the cellular viral load.\(^28,31\)

Once the viral genome is free in the cytoplasm, the translation at the endoplasmic reticulum starts with the synthesis of the pp1a and pp1ab that are subsequently processed to produce the nsps. The latter, and particularly nsps 3, 4, and 6, induce the formation of a double-membrane vesicle (DMV) from deranged membranes of the endoplasmic reticulum.\(^31\) Also, the 3’-terminal of the viral genome is translated to produce the S, E, M, N, and accessory proteins. Meanwhile, the RNA genome is replicated. DMV is the platform where the virus assembles.\(^28\) It has been reported that CoV particles egress the cell via the conventional secretory pathway passing through the Golgi apparatus. Yet, it seems that β-CoV virions are preferentially de-routed into the endosomal-lysosomal system and then secreted via calcium-dependent lysosome exocytosis.\(^34\)

The connection between β-CoV infection/replication and autophagy was first recognized in studies on Mouse Hepatitis Virus.
(MHV), Infectious Bronchitis Virus (IBV), Middle East Respiratory Syndrome (MERS-CoV), and Severe Acute Respiratory Syndrome-CoV-1 (SARS-CoV-1). Intriguingly, the DMV used for β-CoV assembly resembles the structure of the autophagosome, although it is smaller in size (<0.3 μm) and contains LC3-I, but not LC3-II. Both canonical and noncanonical autophagy pathways are triggered by and play a role in CoV infection and replication. Interestingly, knock-down of either ATG5, ATG7, or BECLIN1 did not abrogate (or it could improve) CoVs replication in cultured cells (reviewed in), suggesting that noncanonical autophagy probably plays a major role. To be noted, the actual role of macroautophagy in viral infection is double-faced, as it can either promote or inhibit viral replication, the outcome likely reflecting the cell type (with its genetic and epigenetic background) and the surrounding microenvironment that together impinge on the regulation of autophagy. Additionally, viral proteins impact the autophagy-lysosomal machinery as briefly illustrated below. Particularly, like other virus families, CoVs have evolved strategies to hijack autophagy at different steps to benefit from its stimulation or inhibition while avoiding degradation. For instance, human SARS-CoV nsp6 stimulates autophagosome formation and at the same time, it limits autophagosome expansion and fusion with lysosomes, thus impairing autophagy clearance efficiency. The combined effect is that autophagosomal membranes accumulate and could be retrieved for the generation of DMV, where the virus assembles. Further, it has been hypothesized that such an effect in antigen-presenting cells would compromise the capability of autophagosomes to deliver viral components to lysosomes for degradation as well as reducing antigen presentation and/or exposure to TLRs. In line with this finding, it has been shown that membrane-associated papain-like protease PLP2 (PLP2-TM) of SARS-CoV interacts with BECLIN1 and promotes the accumulation of autophagosomes through impairment of autophagosome-lysosome fusion. In the same line, MERS-CoV replication was associated with proteasomal degradation of BECLIN1 and impaired fusion of autophagosomes and lysosomes. Inhibiting BECLIN1 degradation restored autophagy and drastically reduced MERS-CoV replication, implying that stimulation of autophagy to completion could be a strategy for limiting the viral output.

The mechanistic interaction between CoV entry, replication, and exit processes with the endocytic, autophagy, and endosomal-lysosomal system is schematized in Fig. 2. Taken together, the studies on the five better known CoVs, namely IBV, MHV, MERS, SARS-CoV-1, and SARS-CoV-2, indicate that during their infection and propagation these viruses interact with the autophagy-lysosomal system at three levels, and precisely: (i) when they enter via endocytosis and exploit endosomal cathepsin L; (ii) when they induce autophagosomes and meanwhile impair autolysosome formation, thus escaping from degradation and exploiting autophagosomal membranes for the construction of DMV; and (iii) when they exit via the unconventional lysosomal secretory pathway.

The relationship between autophagy and viral infection also includes the participation of autophagy in innate and adaptive immunity and modulation of the inflammatory response. Auto-phagy plays a major role in viral antigen processing and priming of CD4+ and CD8+ T-lymphocytes that is instrumental for humoral and cellular response to virus infection. Auto-phagy also dampens inflammation. Lack of ATG5 in dendritic cells resulted in increased secretion of proinflammatory cytokines upon respiratory syncytial virus infection. Similarly, the lack of RUBICON, a BECLIN-1 interacting protein essential in the non-canonical autophagy pathway LAP, resulted in significant production of IL-6, IL-1β, and IL-12 upon viral infection.
In the next paragraph, we present the natural products that have the potential to interfere with the autophagy-dependent infection and replication of the main human pathogenic viruses. We discuss their mechanism of action in light of similarities with SARS-CoV-2, and aim to provide additional supporting information for the development of new drugs for the management of the current pandemic.

4. Natural products targeting autophagy to halt cell-to-cell virus propagation

Herbs and natural compounds interfere in different stages of the viral cycle and exert a supportive role on the host immune response for clearing viral infections.53 For instance, berberine, baicalin, and resveratrol inhibited the human immunodeficiency virus (HIV), influenza A virus (IAV), human cytomegalovirus (HCMV), and many others through different pathways.54–56 Here, we present a selection of herbs and phytochemicals that specifically explored autophagic mechanisms to counteract the infection and replication of RNA viruses such as enterovirus-71 (EV71), influenza viruses (H1N1, H3N2, H5N1, H9N2), and HCV. Also, we extended our search by looking at the in silico evidence of each herb-compound for its possible activity toward SARS-CoV-2 infection (Tables 1 and 2).

4.1. Anti-SARS-CoV-2 activity of berberine

Berberine (BBR) is an isoquinoline alkaloid present in several herbal species such as Coptidis rhizome, Phellodendron chinese, and Berberis vulgaris.56 BBR was able to contrast the influenza virus H1N1 infection through stimulation of the autophagy flux and mitophagy, as indicated by the upregulation of BNIP3 and LC3-II expression.57 Consequently, BBR mitigated the mitochondrial production of Reactive Oxygen Species (ROS) and the activation of the NLRP3 inflammasome, and reduced activation of caspase-1 and secretion of IL-1β, thus improving pulmonary inflammatory lesions, edema, and hemorrhage in pneumonia-induced influenza.57 Additionally, BBR was shown to prevent enterovirus 71 replication by inhibiting the virus-mediated induction of autophagy, which consistently suggests that induction of autophagosome formation was instrumental to virus biogenesis and assembly.58

Molecular docking and dynamics simulations revealed that BBR significantly interacts with SARS-CoV-2 3CLpro,59 S protein, and ACE2 receptor59–61 (Table 1). This suggests that BBR could prevent viral entry and fusion with the autophagy processes and the biogenesis of DMV by affecting the 3CLpro-mediated generation of nsps 4–16.62 Accordingly, BBR potently inhibited SARS-CoV-2 replication.63,64 Advantages of using BBR include the reported low cytotoxicity and high cellular viability, prevention of infection at low concentrations, no serious adverse effects, and easy penetrability in various organ systems (e.g., liver, kidneys, muscles, and brain).65,66 Disadvantages to its use include poor aqueous solubility and rapid metabolism. Yet, this issue can be resolved by using nanoparticles and lipid-based nanocarriers.67

4.2. Anti-CoV activity of baicalin and baicalein

Baicalin, a flavonoid extracted from Scutellaria baicalensis can hinder the formation of autophagosomes and inhibit the H3N2-induced autophagy by counteracting the mTOR suppression in the infected epithelial and macrophage cells.68 Also, baicalin downregulated the expression of LC3-II, ATG5, and ATG12, and this led to a decreased virus replication.69 Baicalein may interact with the SARS-CoV-2 S and PLpro, nsp4, and 3CLpro proteins,70–72 suggesting another way to prevent the induction of autophagy. Notably, the in silico evidence indicates the potential of baicalein, baicalin, and some baicalin derivatives to block 3CLpro,73 baicalin to counteract nsp4, nsp15, nsp16, RdRp, and furin,74 and baicalein to inhibit nsp14 N and C terminals.75

4.3. Anti-SARS-CoV-2 activity of resveratrol

Resveratrol (RV) is a naturally occurring stilbene molecule of the polyphenol family present in plants such as Polygonum cuspidatum, Panax pseudoginseng, Smilax glabra, and Aloe vera L. var. chinensis.76 RV effectively decreased the synthesis of EV71 capsid protein VP1 and virus replication along with attenuation of oxidative stress and inhibition of cytokines IL-6, IL-8, and TNF-α secretion.77,78 Also, RV reduced the expression of N protein and contrasted virus-induced apoptosis in MERS-CoV-infected cells.79 In vitro studies show that RV may be effective at suppressing CoVs including the SARS-CoV-2 with low cytotoxicity while maintaining cell viability even at high concentrations.79–81 In silico analysis demonstrated the ability of RV and its derivatives to strongly and stably block SARS-CoV-2 proteins PLpro and RdRp, and S protein.79–81 RV could act as an ACE2 receptor inhibitor, preventing the formation of the S1/ACE2 complex and viral endocytosis, and DMV biogenesis. Furthermore, RV could impact the autophagic process through inhibition of PLpro-mediated generation of nsps. These actions make RV an interesting candidate for the treatment of SARS-CoV-2 infection.

4.4. Anti-SARS-CoV-2 properties of Catechin

Catechins are major polyphenol compounds found in green tea leaves. There are five major types of catechins, namely catechin, epicatechin, epigallocatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate.82 Catechin decreased the viral load of influenza A virus (IAV) H1N1 in a dose-dependent manner and protected the infected cells, and this effect was associated with inhibition of H1N1 M2 and NP proteins and downregulation of autophagy.83 Catechin interfered with SARS-CoV-2 infection and replication by neutralizing 3CLpro, S protein RBD, ACE2, S/ACE2 complex, cathepsin L, nsps, and N protein.84–86 Tea polyphenols, including epigallocatechin-3-O-gallate (EGCG) and theaflavin 3,3’di-gallate, can strongly dock to 3CLpro, S protein, S/ACE2 complex, PLpro, and RdRp.84–85 Also, a computational analysis revealed that several compounds with a common catechin skeleton can inhibit SARS-CoV-2 protein N.84 Altogether, these compounds could suppress the SARS-CoV-2 replication through the inhibition of viral proteins involved in DMV biogenesis and autophagy. However, green tea polyphenols have low bio-accessibility (90% is lost upon gastric and intestinal digestion) and low bioavailability. Therefore, more research is needed to inform clinical applications of catechins for CoVs.

4.5. Anti-SARS-CoV-2 properties of procyanidins

While catechins are monomers of flavan-3-ol, procyanidins are oligomers and polymers of flavan-3-ol units found especially in Vaccinium angustifolium Ait (blueberry), Vitis vinifera L. (grape seed), and Cinnamomum cassia Presl (cinnamon bark).87 Procyanidin can significantly inhibit IAV replication in a concentration-dependent manner with no cytotoxicity.88 Importantly, procyanidin’s antiviral mechanism is associated with reduced production and accumulation of LC3-II and decreased expression of ATG5, ATG7, and ATG12.89 Additionally, procyanidin inhibited the formation of ATG5-ATG12/ATG16 heterotrimer and stabilized the BECLIN1/BCL-2 heterodimer.90 These data support the idea that the antiviral property of procyanidin relies on its ability to inhibit the autophagy process. Procyanidin A2 and B1 have shown moderate anti-SARS-CoV activity.91 The latest research has evidenced the...
The influence of herbs-compounds on autophagy in RNA viral models and their potential to counteract autophagy through SARS-CoV-2/host protein inhibition. The second column provides a summary of autophagy markers after the intervention of natural products. The third column shows the interaction between SARS-CoV-2/host proteins and herbs-compounds. Herbs and compounds that block proteins implicated in the autophagic process may be able to inhibit viral entry, fusion, and endocytosis (S protein, ACE-2, S/Ace-2 complex, Furin, TMPRSS2 receptor, Cathepsin-L (CTSL)) and autophagosome and DMV biogenesis (nsp3/PLpro, nsp4, nsp6, nsp8, 3CLpro, and possibly nsp2). Some of the herbs-compounds are multi-target proteins and could interfere in different phases of the viral life cycle. Note that, in the in silico studies, researchers have different opinions on the docking score levels able to strongly and stably block proteins and markers. Thus, some scores may be considered appreciable as protein inhibitors while others may be considered weak. NOTE: references to Table 1 are listed in supplementary file.

| Herb or phytocompound | Autophagic mechanisms/markers in response to herbs or phytocompounds in RNA viral models | SARS-CoV-2/host proteins (in silico studies – potential inhibition action of herbs-compounds) |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Berberine (BBR)       | Autophagy induction (H1N1) – *in vitro and in vivo animal models*<br> 1. BNIP3<br> 2. MMP<br> 3. LC3-II<br> 4. p62<br> 5. mTOR<br> 6. caspase-1<br> 7. IL-1β<br> 8. NLRP3 (Liu et al., 2020) | - **SARS-CoV-2**: 3CL<sup>pro</sup>: 83.2 kcal/mol<br> 5. S protein: 69.70 kcal/mol<br> 6. Furin: 71.50 kcal/mol (Lakshmi et al., 2020) |<br>- **SARS-CoV-2**: 3CL<sup>pro</sup>: 7.3 kcal/mol (Chowdhury et al., 2020) |<br>- **Immunotherapeutic-Berberine nanomedicine (NIT-X)**<br> MAPK3: 8.9 kcal/mol<br> MAPK8: 8.6 kcal/mol<br> TNF-α: 8.2 kcal/mol<br> MAPK1: 8.2 kcal/mol<br> BAX: 8.1 kcal/mol<br> NF-κB1: 7.3 kcal/mol<br> CHIK: 7.3 kcal/mol<br> ACE-2 receptor: 9.8 kcal/mol (Wang et al., 2020) |
| Baicalin               | Autophagy inhibition (H3N2) –<br> 1. mTOR<br> 2. LC3-II/GAPDH ratio<br> 3. Atg5-Atg12 (Zhu et al., 2015) | - **SARS-CoV-2**: 3CL<sup>pro</sup>: 8.458<br> 1. S protein: 8.277<br> 2. Hesperidin: 2.0 kcal/mol<br> 3. Baicalein: 6.8 kcal/mol<br> 4. Withaferin-A: 9.3 kcal/mol<br> 5. Berberine (BBR and its derivative 2d) |<br>- **SARS-CoV-2**: 3CL<sup>pro</sup>: 6.78 kcal/mol<br> 1. S protein: 8.738<br> 2. Catechin - SARS-CoV-2: 8.776<br> 3. Baicalin: 8.776 |<br>- **SARS-CoV-2**: Nsp14 N-terminal and C-terminal: 8.7 kcal/mol (Liu et al., 2020) |<br>- **SARS-CoV-2**: Nsp14 C-terminal: 8.7 kcal/mol (Liu et al., 2020) |<br>- **SARS-CoV-2**: Nsp16: 8.6 kcal/mol (Chandra et al., 2021) |
| Resveratrol (RES) and RESloaded nanoparticles | Autophagy inhibition (EV71) –<br> 1. p-ERK, P-Ef2, ATP4, GRP78, and CHOP<br> 2. LC3-II<br> 3. LC3-II/LC3-I ratio<br> 4. IL-6, IL-8, and TNF-α<br> 5. MDA and ROS<br> 6. SQSTM1/p62<br> 7. MAPK1<br> 8. JNK | - **RES and SARS-CoV-2 PL<sup>pro</sup> (6W9C) and RdRp (6M71)** - strongest inhibitions:<br> RV-13 (PL<sup>pro</sup> - 184.99 kj/mol)<br> RV-12 (RdRp - 173.76 kj/mol) (Ranjbar et al., 2020) |<br>- **RES**: Most stable interaction with 51/ACE-2 complex: 8.0 kcal/mol (Wahedi et al., 2020) |<br>- **RES**: -O-ribose methyltransferase (MTase) nps16: 8.7 kcal/mol (Chandra et al., 2021) |<br>- **RES**: -O-ribose methyltransferase (MTase) nps17: 6.78 kcal/mol (Chandra et al., 2021) |<br>- **RES**: 3CL<sup>pro</sup>: 6.78 kcal/mol (Chandra et al., 2021) |
| Catechin (C) and Tea Polyphenols | Autophagy inhibition (H1N1) –<br> 1. M2<br> 2. NP<br> 3. LC3B<br> 4. P38K-III (Chang et al., 2020) | - **Catechin - SARS-CoV-2**: 3CL<sup>pro</sup>: 8.34 kcal/mol<br> 1. CTL: 7.68 kcal/mol<br> 2. S protein RBD: 5.79 kcal/mol<br> 3. N protein: 6.23 kcal/mol<br> 4. S protein RBD-EGCG: 8.3 kcal/mol<br> 5. S protein RBD-EGCG: 8.4 kcal/mol<br> 6. S protein RBD-EGCG: 9.7 kcal/mol |
Table 1 (continued)

| Herb or phytocompound | Autophagic mechanisms/markers in response to herbs or phytocompounds in RNA viral models | SARS-CoV-2/host proteins (in silico studies – potential inhibition action of herbs-compounds) |
|-----------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Procyanidins          | Autophagy inhibition (IAV)                                                            | · **Procyanidins and Catechins - SARS-CoV-2 3CL\textsuperscript{pro}:**                      |
|                       | · LC3-II/\beta-actin ratio                                                             | Procyanidin B2 (PB2): 9.2 kcal/mol                                                       |
|                       | · LC3-II accumulation                                                                 | Procyanidin A2 (PA2): 9.2                                                               |
|                       | · LCB3-I to II conversion                                                              | (--)-epigallocatechin-3\textsuperscript{-}O-gallate (EGCG): 8.7                         |
|                       | · Atg7, 5, and 12 expression                                                            | (--)-gallocatechin-3\textsuperscript{-}O-gallate (ECG): 8.7                            |
|                       | · Atg5-Atg12/Atg16 heterotrimer                                                         | (--)-epicatechin-3\textsuperscript{-}O-gallate (CAG): 8.3                                |
|                       | Stabilized beclin1/Beclin2 heterodimer (Dai et al., 2012)                              | (--)-gallocatechin (GC): 7.5                                                            |
|                       |                                                                                      | (--)-epicatechin (EPC): 7.5                                                            |
|                       |                                                                                      | (+)-catechin-3\textsuperscript{-}O-gallate (CAG): 8.3                                   |
|                       |                                                                                      | Lopinavir (LOP): 8.0                                                                    |
|                       |                                                                                      | Ebselen (EBS): 6.6                                                                     |
|                       |                                                                                      | Cinanserin (INN): 5.4                                                                  |
|                       |                                                                                      | (Zhu and Xie, 2020)                                                                    |
|                       |                                                                                      | Procyanidins: **Theaflavin, and Theasinsensins:**                                       |
|                       |                                                                                      | RdRp: **Theaflavin 3,3’di-gallate (TF3): 14.92**                                         |
|                       |                                                                                      | Theaflavin 3’-gallate (TF2a): 13.26                                                      |
|                       |                                                                                      | Digalloylprocyanidin B2: 13.26                                                          |
|                       |                                                                                      | **3CL\textsuperscript{pro},**                                                           |
|                       |                                                                                      | Procyanidin: 11.68                                                                      |
|                       |                                                                                      | TF2a: 11.52                                                                            |
|                       |                                                                                      | Theasinensin A: 11.07                                                                  |
|                       |                                                                                      | Theasinensin B: 10.73                                                                  |
|                       |                                                                                      | (Gogoi et al., 2021)                                                                   |
|                       |                                                                                      | · **Procyanidins:**                                                                    |
|                       |                                                                                      | Nsp1: 8.5 kcal/mol                                                                     |
|                       |                                                                                      | Nsp2: 8.8 kcal/mol                                                                     |
|                       |                                                                                      | Nsp3: 9.7 kcal/mol                                                                     |
|                       |                                                                                      | Nsp4: 8.9 kcal/mol                                                                     |
|                       |                                                                                      | Nsp6: 8.1 kcal/mol                                                                     |
|                       |                                                                                      | Nsp7: 8.7 kcal/mol                                                                     |
|                       |                                                                                      | Nsp8: 8.4 kcal/mol                                                                     |
|                       |                                                                                      | Nsp10: 8.3 kcal/mol                                                                    |
|                       |                                                                                      | RdRp: 8.9 kcal/mol                                                                     |
|                       |                                                                                      | Helicase: 9.5 kcal/mol                                                                  |
|                       |                                                                                      | ExonN: 9.6 kcal/mol                                                                     |
|                       |                                                                                      | Nendol: 8.2 kcal/mol                                                                    |
|                       |                                                                                      | Z’-O-MT: 10.1 kcal/mol                                                                   |
|                       |                                                                                      | ORF3a: 7.9 kcal/mol                                                                     |
|                       |                                                                                      | M protein: 7.8 kcal/mol                                                                  |
|                       |                                                                                      | ORF6: 6.7 kcal/mol                                                                      |
|                       |                                                                                      | ORF7a: 8.0 kcal/mol                                                                     |
|                       |                                                                                      | ORF8: 8.9 kcal/mol                                                                      |
|                       |                                                                                      | N protein: 8.7 kcal/mol                                                                  |
|                       |                                                                                      | ORF10: 7.2 kcal/mol                                                                     |
|                       |                                                                                      | ACE-2: 8.9 kcal/mol                                                                     |
|                       |                                                                                      | M\textsuperscript{pro}: 9.2 kcal/mol                                                     |
|                       |                                                                                      | S protein: 9.5 kcal/mol                                                                  |
|                       |                                                                                      | · **Quercetin-7-0-glucoside (Q7G):**                                                     |
|                       |                                                                                      | · Acidic Vesicular Organelles (AVO)                                                      |
|                       |                                                                                      | · Atg-5, Atg-7                                                                          |
|                       |                                                                                      | · LCB-3                                                                                |
|                       |                                                                                      | · ROS (Gansukh et al., 2016)                                                           |
|                       |                                                                                      | · S RBD-TF3: 11.6 kcal/mol                                                              |
|                       |                                                                                      | PL\textsuperscript{pro}, EGCC: 8.9 kcal/mol                                              |
|                       |                                                                                      | PL\textsuperscript{pro}, TF3: 11.3 kcal/mol                                              |
|                       |                                                                                      | RdRp-EGCC: 5.7 kcal/mol                                                                 |
|                       |                                                                                      | RdRp-TF3: 6.0 kcal/mol                                                                   |
|                       |                                                                                      | ACE2/S RBD-EGCC: 8.5 kcal/mol                                                           |
|                       |                                                                                      | ACE2/S RBD-TF3: 8.0 kcal/mol                                                            |
|                       |                                                                                      | (Mhatre et al., 2021)                                                                   |
|                       |                                                                                      | Procyanidins Autophagy inhibition (IAV)                                                  |
|                       |                                                                                      | · LC3-II/\beta-actin ratio                                                              |
| Quercetin-7-0-glucoside (Q7G) | Autophagy inhibition (IAV) – \textit{in vitro}                                      | · Acidic Vesicular Organelles (AVO)                                                      |
|                       |                                                                                      | · Atg-5, Atg-7                                                                          |
|                       |                                                                                      | · LCB-3                                                                                |
|                       |                                                                                      | · ROS (Gansukh et al., 2016)                                                           |
Table 1 (continued)

| Herb or phytocompound | Autophagic mechanisms/markers in response to herbs or phytocompounds in RNA viral models | SARS-CoV-2 host proteins (in silico studies – potential inhibition action of herbs-compounds) |
|-----------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| **Saikosaponins (Ss)** (SsA and SsD) | **Autophagy inhibition (EV71) – in vitro**  
  - Activation of RAB-5 > defects in lysosome biogenesis and increase lysosomal pH  
  - Lysosomal pH > induces TFEB nuclear translocation  
  - Inhibition of autophagosome-lysosome fusion  
  - p62  
  - No effect on mTOR  
  - Increase lysosomal pH  
  - Activation of RAB-5  
  - p38 MAPK  
  - ERK | **Others:** see original paper (Saakre et al., 2021)  
  - **Saikosaponins and SARS-CoV-2:**  
    - S protein: 8.299 kcal/mol  
    - Other strongest inhibitors: saikosaponin V and U (Sinha et al., 2020)  
  - **Ss and COVID-19 – interaction complexes:**  
    - IL-6/SsU: 6.978 kcal/mol  
    - IL-6/SsV: -7.077 kcal/mol  
  - JAK3-SsA: 7.981 kcal/mol  
  - JAK3-SsD: 7.942 kcal/mol  
  - NOX5-SsA: 7.813 kcal/mol  
  - NOX5-SsD: 2.022 kcal/mol  
  - Best interactions: JAK3-Ss compounds  
  - Saikosaponins interacted with CAT Gene CAT (Catalase) and Checkpoint kinase (CHIK1) (Chikhale et al., 2021)  
  |  
| **Eugenol** | **Autophagy inhibition (IAV) – in vitro**  
  - ERK  
  - p38 MAPK  
  - IKK/NF-κB  
  - Dissociation of Beclin 1-Bcl-2 heterodimer  
  - TNF-α, IL-6, and IL-8 (Dai et al., 2013) | **Eugenol:**  
  - SARS-CoV-2 3CL<sup>pro</sup> (6LU7): 5.4 kcal/mol  
  - S protein (6VXX): 6.1 kcal/mol  
  - EGCG: 7.077 kcal/mol  
  - S protein: 9.8 kcal/mol  
  - 3CL<sup>pro</sup>: 7.8 kcal/mol  
  - mTOR pathway  
  - P38 MAPK  
  - ERK  
  |  
| **PGG** | **Autophagy inhibition (RABV) – in vitro and in vivo animal model**  
  - LC3-II  
  - SQSTM1/p62  
  - mTOR  
  - Viral absorption and entry  
  - Viral titers  
  - P protein expression  
  - mRNA expression and protein synthesis  
  - Symptoms and mortality (mice) (Tu et al., 2018) | **SARS-CoV-2 3CL<sup>pro</sup>:**  
  - PGG: 6.4 kcal/mol  
  - EGCG: H41 residue: -3.5 kcal/mol and C145 residue: -6.0 kcal/mol (Chiu et al., 2021)  
  - 5 S RBD/ACE-2 complex: 8.0 kcal/mol  
  - Additional analysis showed that PGG may interact with both the S-RDB and ACE-2 receptor, thus preventing their interaction. (Chen et al., 2021)  
  |  
| **Aloe vera** | **Autophagy inhibition (IAV) – in vitro**  
  - Inhibition of IAV-induced autophagy > fluorescence microscopy and Cyto-ID<sup>®</sup> Autophagy Detection Kit  
  - In silico – binding affinities:  
    - Aloe-emodin-M2: -5.47 kcal/mol  
    - Catechin hydrate-M2: -5.48 kcal/mol  
    - Quercetin-M2: -5.35 kcal/mol  
    - Anantadine-M2 (control): -4.52 kcal/mol (Choi et al., 2019)  
  | **Aloe vera molecules – SARS-CoV-2 3CL<sup>pro</sup>:**  
  - Feralolid: 7.9 kcal/mol  
  - 9-dihydroxy-2-(O-2-cinnamyl)-methoxy-aloesia: 7.7 kcal/mol  
  - Aloeresin: 7.7 kcal/mol  
  - Isoaloeresin: 7.3 kcal/mol  
  - Aloin A: 7.1 kcal/mol  
  - Eigionica dimer A: 7.1 kcal/mol  
  - 7-O-methylaloeresin: 7.0 kcal/mol  
  - Chrysophanol: 6.8 kcal/mol  
  - Aloe-eminon: 6.7 kcal/mol  
  - Aloin B: 6.7 kcal/mol (Mpiana et al., 2020)  
  | No articles found  
| **Evodiamine** | **Autophagy inhibition (IAV) – in vitro**  
  - Atg5-Atg12/Atg16 heterotrimer formation  
  - Atg5, Atg12, and Atg16 protein expression  
  - LC3-II and p62 accumulation  
  - AMPK/ATG5/TSC2/mTOR pathway  
  - TNF-α, IL-1β, IL-6, and IL-8 (Dai et al., 2012–2) | No articles found.  
| **Deguelin** | **Autophagy inhibition (HCV) – in vitro**  
  - LC3B-II/LC3B-II (S)-actin  
  - LC3B-I to LC3B-II conversion  
  - p62  
  - Beclin-1 (Liao et al., 2020) | No articles found.  
| **Kurarinone** | **Autophagy inhibition (IAV) – in vitro**  
  - No articles found. | No articles found.  

62
capacity of procyanidins to significantly interact with SARS-CoV-2 3CL\textsuperscript{PRO} - nsp1, nsp2, Pl\textsuperscript{PRO}, nsp4, nsp6, nsp7, nsp8, nsp9, nsp10, Rdrp, helicase, exon N, NendoU, 2'-O-MT, ORF3a, E protein, M protein, ORF6, ORF7a, ORF8, ORF10, ACE-2, S protein, S/ACE2 complex, PL\textsuperscript{PRO}, nsps4, nsps5/3CL\textsuperscript{PRO}, nsp6, and nsp8. This table shows the herbal-protein interactions. For specific docking scores see Table 1.

### 4.6. Anti-SARS-CoV-2 activity of quercetin-7-0-glucoside

Quercetin-7-0-glucoside (Q7G) is composed of a glucosyl residue attached at position 7 of quercetin via a β-glycosidic link. In IAV-infected cells, Q7G prevented IA V replication by blocking viral RNA synthesis, and in parallel prevented the accumulation of acidic vesicular organelles.\textsuperscript{92} However, the labeling of acidic organelles with acridine orange indicates that Q7G only prevented the extension of the potential of RdRp, helicase, exon N, NendoU, 2'-O-MT, ORF3a, E protein, M protein, ORF6, ORF7a, ORF8, ORF10, ACE-2, and S protein.\textsuperscript{94,95} Interestingly, flavan-3-ols and procyanidins were also significantly associated with ACE inhibition rather than stimulation of autophagy in the early stages.\textsuperscript{101} Also, saikosaponins (A and B2) have shown anti-human rhinovirus type 1A (HRV-1A) and 2A activity, and 3CL\textsuperscript{PRO} mediated generation of nsps 4–16. However, further studies are needed to validate this hypothesis.

### 4.7. Saikosaponin (Ss), Eugenol, 1,2,3,4,6-penta-0-galloyl-β-o-glucose (PGG), Aloe vera, evodiamine, deguelin, and Kurarinone: inhibition of virus-induced autophagy

Another series of less discussed herbs and phytocompounds have demonstrated their capacity to inhibit autophagic pathways to suppress RNA viral replication. The triterpenoid saponins saikosaponin A (SsA) and B (SsB) extracted from Bupleurum falcatum and other Bupleurum spp. can activate RAB-5, increase lysosomal pH, inhibit autophagosome-lysosome fusion in EV71-infected HeLa cells resulting in the accumulation of autophagosomes.\textsuperscript{102} This was interpreted as autophagy inhibition rather than stimulation of autophagy in the early stages.\textsuperscript{103} Also, saikosaponins (A and B2) have shown anti-human coronavirus 229E (hCoV-229E) activity in vitro with no toxicity.\textsuperscript{102} SsB, was confirmed as a novel anti-CoV potently hindering the viral attachment and entry during the early stage and detaching the viruses that had already adhered to cells.\textsuperscript{102} Also, SsD is capable of strongly reducing viral RNA replication, protein synthesis, and...
titers with little toxicity while significantly inhibiting EV71-induced cell death. In silico studies have ascertained the capacity of saikosaponins to potently block SARS-CoV-2 S protein and nsp15. Thus, saikosaponins could be valuable resources to fight viral-induced autophagy, reduce inflammation, ROS, necrosis, and cell damage in COVID-19.

Eugenol, the major constituent of Syzygium aromaticum, inhibited IAV replication along with the inhibition of autophagy. One possible mechanism for downregulation of autophagy relies on the ability of Eugenol to inhibit the dissociation of BECLIN1-BCL-2 heterodimer. So far, the research on Eugenol for SARS-CoV-2/COVID-19 has been restricted to a few in silico studies. Eugenol appears to be able to interact with some of the virus/host proteins such as 3CLPRO. S protein, ACE2, nsp15, RdRp, and ADP ribose phosphatase, thus interfering in the viral life cycle by preventing the attachment to target cells, endocytosis, and DMV biogenesis.

PGG (1,2,3,4,6-penta-O-galloyl-β-D-glucose), a simple hydrolysable tannin present in many medicinal plants such as Rhus chinensis Mill and Peonia suffruticosa, demonstrated inhibitory activity against rabies virus (RABV) limiting viral RNA synthesis in infected mice and in vitro. This effect was associated with mTOR-mediated inhibition of autophagy. On the contrary, PGG was active against IAV, limiting the expression of viral M2 and NP proteins through induction of autophagy. In silico studies show that PGG can interact with SARS-CoV-2 3CLPRO and S/ACE2 complex (Supplementary Table 1). Additional in vitro and FRET analyses also demonstrated that PGG may hinder SARS-CoV-1 and SARS-CoV-2. Altogether, PGG could halt SARS-CoV-2 through impeding attachment and endocytosis, and through modulation of the autophagic pathway.

Aloe vera extract can suppress IAV (H1N1 and H3N2)-induced autophagy. In vitro, Aloe vera extract limited the expression of all influenza viral proteins (M1, M2, and HA) in a concentration-dependent manner, and reduced viral mRNA levels. Since suppression of IAV M2 proton channel activity leads to the blockage of autophagosome-lysosome fusion, interactions between M2 and Aloe vera compounds such as aloe-emodin, catechin, and quercetin could potentially provide autophagy-mediated antiviral options for RNA viral infections. Based on the in silico activity against 3CLPRO, Aloe vera molecules may indirectly inhibit DMV formation and SARS-CoV-2 induced autophagy (Table 1). However, Aloe vera and its isolates may inhibit other SARS-CoV-2 structural and non-structural proteins that we are unaware of.

Evodiamine, the major active compound of Evodia rutaecarpa, was shown to inhibit IAV-induced autophagy by either acting on the AMPK/TSC2/mTOR pathway or by suppressing ATG5, ATG12, and ATG16 protein expression. However, the possible inhibitory effects of evodiamine or Evodia rutaecarpa on CoVs infection and replication have not been explored yet.

Deguelin is a rotenoid extracted from plants of the Leguminosae family such as Mundulea sericea (bark), Derris trifoliata Lour (root), Tephrosia vogelli Hook (leaves), and Derris trifoliata (root). Deguelin inhibited the HCV-induced autophagy at its early stage, as demonstrated by downregulation of LC3B-I to LC3B-II conversion and accumulation of p62/SQSTM1. Deguelin also suppressed the expression of BECLIN1 leading to the activation of type I IFN response, which further contributed to the inhibition of viral replication. Whether deguelin can exert similar antiviral activity on CoVs has not been investigated yet.

Kuranitone, a flavanone present in S. flavescens, has demonstrated its ability to inhibit HCoV-OC43 in vitro in a dose-dependent manner (IC50 3.34 μM) at the early stage of infection. This antiviral activity was paralleled by inhibition of autophagy, as indicated by decreased LC3-I/LC3-II conversion and increased level of p62/SQSTM1. As of now, there are no studies on Sophora flavescens and its phytocompounds for SARS-CoV-2.

5. Limitations and perspectives

It is evident that we have a lot more information on natural products that inhibit rather than induce autophagy for contrasting RNA viral infections. Also, some herbs (e.g., berberine) could either promote or inhibit autophagy. It may appear confusing that the reported studies on the antiviral effect elicited by the natural products could be obtained either way, through induction or inhibition of autophagy. One first possible explanation resides in differences in the experimental models such as the type of RNA virus researched, i.e., itsModality of entry in the cell, its replication and egress from the cell, and the genetic background of the cell (that determine how the autophagy stress response is regulated). Importantly, the RNA virus itself can induce autophagy or impair the autophagy flux depending on its stage of replication, i.e., the viral proteins being synthesized and processed. Besides, and most importantly, we have to consider the methodology employed to assess autophagy, which could lead to misinterpretations of the actual role of autophagy. In fact, not all the studies strictly adhered to the guidelines when choosing the markers and the appropriate pharmacologic/genetic manipulations of ATG proteins for monitoring autophagy. Another important limitation when interpreting these data is represented by the stage of the infection at which the involvement of autophagy is investigated. These natural products have a broad action in the regulation of the vesicular traffic that include endocytosis, DMV biogenesis, autophagosome formation and maturation, endosomal-lysosomal secretion, which means they can intervene in all the steps of the viral life cycle. Thus, a natural product as a proper therapeutic agent must be selected in terms of concentration, according to the autophagy developmental stage, and of its effective role in the precise step of virus infection. The timing of administration of the herb-derived antiviral drug acting on autophagy likely impacts the outcome, which could mean either improvement or worsening of the symptoms in COVID-19 patients. This is well illustrated in the case of hydroxychloroquine, the antimalarial alkaloid from Chinchuna repurposed for COVID-19 treatment.

One of the most challenging issues in drug discovery is finding drugs that exert antiviral properties while still preserving cell viability. Besides, cytoxicity, solubility, and bioavailability are also important concerns. This is especially troubling when dealing with viruses that can rapidly cause tissue damage, lung fibrosis, oxidative stress, and impaired tissue function. Many of the herbs reviewed here exerted anti-SARS-CoV-2 action at low concentrations, with acceptable bioavailability, and minimum or no cytotoxicity. Nonetheless, low bioavailability could be overcome through the inclusion of the natural biomolecules in lipid-based nanoparticles. Since viruses need to import or synthesize lipid constituents to make DMVs, we theorize that herbal-loaded lipid-nanocarriers, especially those that target nsps involved in the DMV biogenesis and inhibition of the autophagy flux, could be a smart way to easier penetrate cells, modulate autophagy, and hinder SARS-CoV-2. However, since CoVs can create a variety of strategies to use or mimic the host autophagic machinery to replicate, uncovering unexplored autophagy signaling pathways and mechanisms involved in the SARS-CoV-2 infection may open other possibilities for the use of phytocompounds.

Although the SARS-CoV-2 mutations are happening much more slowly than HIV and influenza virus, researchers detected 12,706 mutations in its genome, the majority being single nucleotide polymorphisms (SNPs) (reviewed in17). Viral mutations can be neutral, beneficial, or deleterious. So far, the majority of mutations
in the SARS-CoV-2 genome are considered neutral, and most of them involve the nsp6, nsp11, nsp13, and S protein (reviewed in \(^{117}\)). The role of SARS-CoV-2 structural and non-structural protein mutations on the autophagic process is still uncertain.\(^{118}\) As autophagy is a double-edged sword, it is unclear, for example, if nsp6 mutations would favor viral replication and evasion from the host immune response, or if it would counteract it.\(^{118}\) However, the main mutation of the D614G variant is at the interface between the individual spike protomers, and not in the RBD of S protein.\(^{117,119}\) Therefore, the herbs and compounds seen to block the S protein RBD are still potentially useful to impede adhesion and halt virus-induced autophagy. As the mutations continue to happen, their interference in the autophagic machinery and the usefulness of herbs and compounds depend on our understanding of these same mutations.

6. Concluding remark

The interaction between autophagy, either canonical and non-canonical, and virus infection is complex and may result in: (i) the virus is effectively degraded via autophagy (virophagy) or (ii) the virus de-regulates the process and uses the autophagy machinery for its replication and egression from the cell.\(^{120}\) Several drugs targeting autophagy have been repurposed as possible therapeutics for COVID-19.\(^{2,121}\) Here, we reported the literature data on natural products that showed an effect on the autophagy process in RNA viral infections. Based on the similarity among RNA viruses, and the research of these herbs for SARS-CoV-2, we hypothesize that they may also work for SARS-CoV-2. Yet, extensive additional research is necessary to validate in vivo this hypothesis.

In support of our hypothesis, we also associated, first hand, the results of the in silico research of herbs and natural compounds for SARS-CoV-2 and how intervention on the reported target proteins could hinder attachment, fusion, endocytosis, and DMV biogenesis and consequently inhibit virus-induced autophagy (Fig. 3).

Thus, in silico research could provide important hints for research on target proteins and autophagic pathways for viral infections. In this line, a recent review uncovered the capacity of artemisinin derivatives to block SARS-CoV-2/host proteins such as artesunate (3CL\(^{\text{PRO}}\), E protein, helicase, N protein, and nsp3, 10, 14, and 15), artemisinin (3CL\(^{\text{PRO}}\), GRP78 receptor), artemether (N protein, helicase, nsp10, and nsp15), MOL736 (cathepsin-L), artelinic acid (S protein), arteannuin B (N protein), and artenimol/DHA (N protein).\(^{122}\) As the strongest inhibition were attained by artesunate and artemisinin, it gives us a hint that they may potentially impede DMV biogenesis and autophagy, and hinder viral replication pointing to potential future research. Interestingly, a recent in vitro study reported the suppression of SARS-CoV-2 and two of its variants (UK B1.1.7 and South Africa B1.351) by the \(A.\ annua\) hot water leaf extract.\(^{123}\) In this study, artemisinin was not the main antiviral agent, while artesunate, artemether, and dihydroartemisinin were deemed ineffective or cytotoxic at elevated concentrations.\(^{123}\) Likely, the viral inhibition was due to the combined components present in the plant, their great bioavailability, and yet unexplored mechanisms (e.g., autophagy). Ultimately, the treatment goal would be to hand back the control of the autophagy machinery to the host no matter the disease stage, while also exerting other biological properties such as anti-inflammatory, antioxidant, anti-pyretic, analgesic, and anti-pulmonary fibrosis. In this line, it is worth mentioning that phytochemicals elicit an anti-inflammatory activity through modulating autophagy in stromal cells as well, including fibroblasts and immune cells.\(^{10}\) Accordingly, one of the

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Fig. 3. Potential autophagic-inhibiting properties of herbs and natural compounds in SARS-CoV-2/COVID-19. The in silico research on herbs-compounds that may target SARS-CoV-2 proteins involved in autophagy and vesicle-dependent viral entry and replication. For all docking scores, see Table 1.
attractive advantages of deepening the knowledge of autophagic pathways for SARS-CoV-2/COVID-19 is the direct modulatory therapeutic interventions on the host rather than acting on the virus, which is prone to mutations over time.

Author's contribution

All authors worked in a coordinated and integrated manner. Specifically, CV and AFu: literature searching and drafting of chapters 4, limitations and discussion; SF: literature searching and drafting of chapters 4, limitations and discussion; C. Vidoni, A. Fuzimoto, A. Ferraresi et al. Journal of Traditional and Complementary Medicine 12 (2022) 55-68.

Declaration of competing interest

Nothing to declare.

Acknowledgements

CV is supported with a post-doctoral fellowship from Universitá degli Studi Magna Graecia (Catanzaro, Italy). AFu is recipient of a post-doctoral fellowship “Paolina Troiano” (id. 24094) granted by Associazione Italiana per la Ricerca sul Cancro (AIRC, Milan, Italy). Thanks to Associazione per la Ricerca Medica Ippocrate-Rhazi (ARM-IR, Novara, Italy) for support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcm.2021.10.003.

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