Journal of Traditional and Complementary Medicine 12 (2022) 55–68

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

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

Chiara Vidoni [a, 1], Andréa Fuzimoto [b, 1], Alessandra Ferraresi [a], Ciro Isidoro [a, *]

[a] Università del Piemonte Orientale, Department of Health Sciences, Novara, Italy
[b] Holistic Sync, Holistic Integrative Medicine, USA

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
Virophagy
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.

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 biomolecules 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.[3,7–10] 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

* Corresponding author. Dipartimento di Scienze della Salute, Università "A. Avogadro", Via P. Solaroli 17, 28100, Novara, Italy.
E-mail address: ciro.isidoro@med.uniupo.it (C. Isidoro).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.
[1] These two authors contributed equally and should either be considered as first author.

https://doi.org/10.1016/j.jtcme.2021.10.003

Abbreviations

ATG	autophagy-related
BBR	berberine;
CL^{PRO}	chymotrypsin-like protease
COVID-19	Corona Virus Disease 2019
CoV	coronavirus
DMV	double-membrane vesicle
E	envelope
EGCG	epigallocatechin-3-O-gallate
EV71	enterovirus-71
hACE2	human angiotensin-converting enzyme 2
hCoV-229E	human coronavirus 229E
IAV	influenza A virus
IBV	infectious bronchitis virus
M	membrane
MAP-LC3	microtubule-associated protein-light chain 3
MERS-CoV	middle east respiratory syndrome
MHV	mouse hepatitis virus
N	nucleocapsid
nsps	non-structural proteins
ORF	open reading frames
PGG	1,2,3,4,6-penta-O-galloyl-β-D-glucose
$PL1^{PRO}$ and $PL2^{PRO}$	papain-like proteinases
pp	polyproteins
Q7G	quercetin-7-0-glucoside
RABV	rabies virus
RBD	receptor binding domain
RdRp	RNA-dependent RNA polymerase
ROS	reactive oxygen species
RTC	replication-transcription complex
RV	resveratrol
S	spike
SARS	severe acute respiratory syndrome
SARS-CoV	severe acute respiratory syndrome-CoV
Ss	saikosaponin
TF3	theaflavin 3,3'di-gallate
TLR	Toll like receptor
TMPRSS-2	transmembrane protease serine protease 2
UTR	untranslated region

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 to form 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 certain ATG proteins (including ATG4, ATG5, ATG7, and ATG12) to be conjugated to phosphatidylethanolamine and thereafter be inserted into the bilayer of both the inner and outer membrane of the autophagosome. This vacuolar form of LC3 is known as isoform LC3-II. Second, while the autophagosome is under construction, the autophagy substrate (e.g., protein aggregates) to be degraded is bound by the p62/SQSTM1 protein and sequestered in 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 amphysome and autolysosome, respectively. The acidic pH and the hydrolytic enzymes (especially, cathepsins) ensure the complete digestion of the material within the autolysosome, from which the elementary 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 ULKC1. Downstream to ULKC1 is the Vps15-BECLIN1-PI3KC3-ATG14L 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 MEK/ERK pathway[20] and a MAPK/JNK pathway.[21] Both canonical and non-canonical 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]

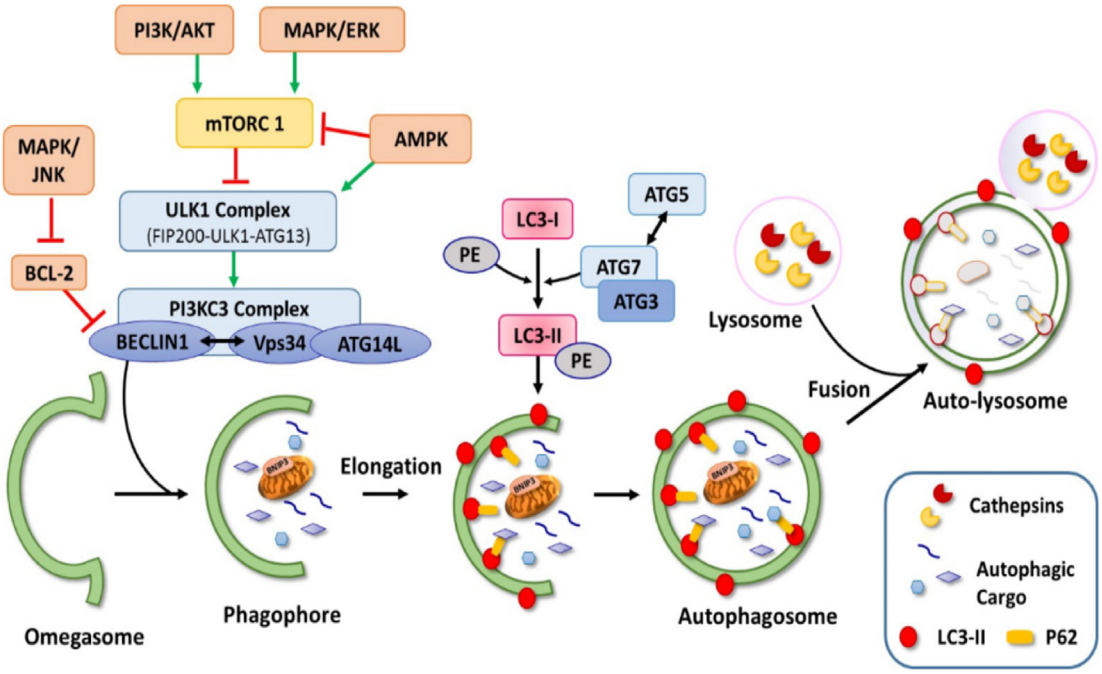

Fig. 1. Autophagy pathway. A schematic overview of the "canonical" pathway, and the main autophagy-related proteins and regulators: the picture shows the three steps starting from the phagophore formation, elongation, and maturation of autophagosome, until the autophagosome-lysosome fusion. The ATG proteins dispensable in the non-canonical pathway are light blue colored. For details refer to the text. ATG: autophagy-related gene or protein.
→: activation or induction; : inhibition; ↔ : interaction.

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/1 ab) 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 ($3CL^{PRO}$; aka Main protease M^{PRO}) and papain-like proteinases ($PL1^{PRO}$ and $PL2^{PRO}$) and in genome replication[27] (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 nsp3, 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.[28,29] 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 obligated 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.[33] 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

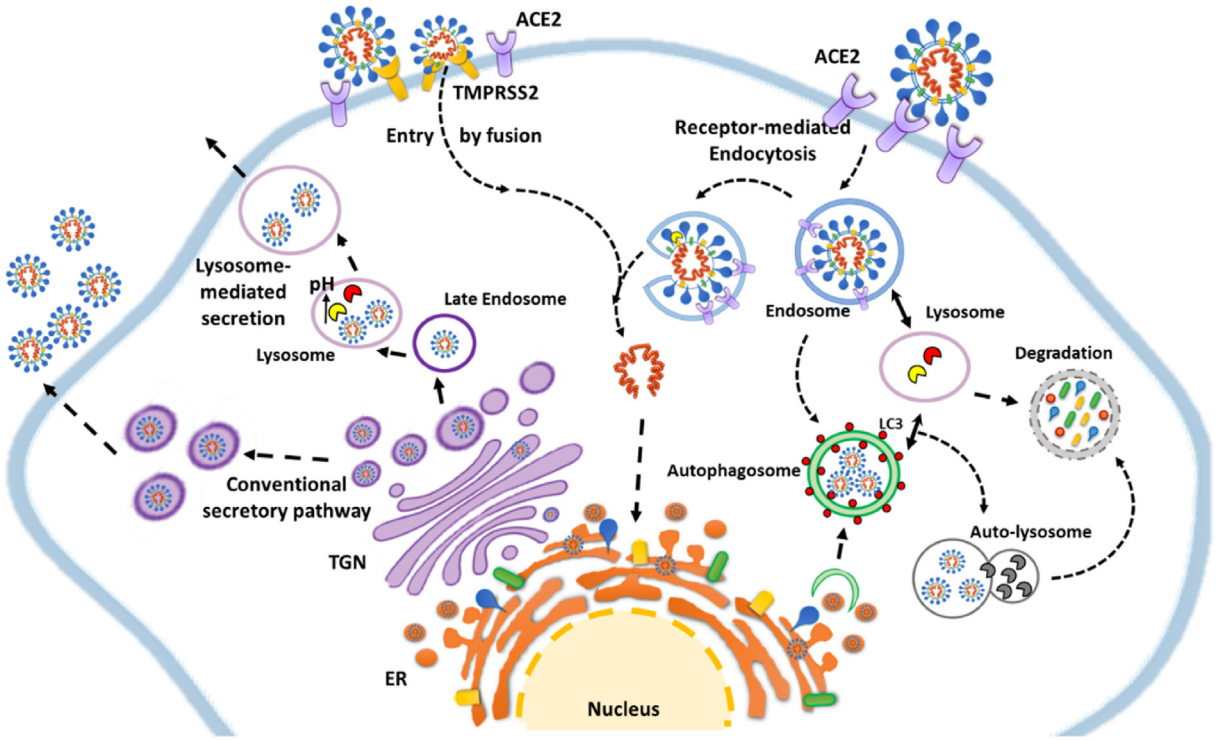

Fig. 2. Cellular mechanistic interaction between coronavirus and endocytic-autophagy-lysosomal compartment. The cartoon shows an overview of strategies related to the modulation of autophagy during the entry (receptor-mediates endocytosis and fusion), viral replication and exit of virions (conventional secretory pathway and lysosome-mediated secretion). For details refer to the manuscript. TGN = Trans Golgi Network; ER = Endoplasmic reticulum; ACE2 = angiotensin-converting enzyme 2.
→ and -→ = promotion; ↔ = interaction.

(MHV), Infectious Bronchitis Virus (IBV), Middle East Respiratory Syndrome (MERS-CoV), and Severe Acute Respiratory Syndrome-CoV-1 (SARS-CoV-1).[35] 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.[36] Both canonical and noncanonical autophagy pathways are triggered by and play a role in CoV infection and replication.[37] Interestingly, knock-down of either ATG5, ATG7, or BECLIN1 did not abrogate (or it could improve) CoVs replication in cultured cells[38] (reviewed in[37]), 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,[39] 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.[39] 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.[10,40,41] The combined effect is that autophagosomal membranes accumulate and could be retrieved for the generation of DMV, where the virus assembles.[41,42] 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.[41] 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.[43] In the same line, MERS-CoV replication was associated with proteasomal degradation of BECLIN1 and impaired fusion of autophagosomes and lysosomes.[44] Inhibiting BECLIN1 degradation restored autophagy and drastically reduced MERS-CoV replication,[44] 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.[45] Autophagy 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.[39,46] Autophagy also dampens inflammation. Lack of ATG5 in dendritic cells resulted in increased secretion of proinflammatory cytokines upon respiratory syncytial virus infection.[47] 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.[48]

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.[49] 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.[49–55] Here, we present a selection of herbs and phytocompounds 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 $3CL^{PRO}$, S protein, and ACE2 receptor[59–61] (Table 1). This suggests that BBR could prevent viral entry and fusion and interfere with the autophagy processes and the biogenesis of DMV by affecting the $3CL^{PRO}$-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 penetration in various organ systems (e.g., liver, kidneys, muscles, and brain).[63,65] Disadvantages to its use include poor aqueous solubility and rapid metabolism. Yet, this issue can be resolved by using nanoparticles and lipid-based nanocarriers.[65]

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.[66] Also, baicalin downregulated the expression of LC3-II, ATG5, and ATG12, and this led to a decreased virus replication.[66] Baicalin may interact with the SARS-CoV-2 S and PL^{PRO}, nsp4, and $3CL^{PRO}$ proteins,[3,34,67,68] 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 $3CL^{PRO}$[3,34,69,70], baicalin to counteract nsp4, nsp15, nsp16, RdRp, and furin,[66,67,71] and baicalein to inhibit nsp14 N and C terminals.[72]

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 *Polygonun cuspidatum, Panax pseudoginseng, Smilax glabra,* and *Aloe vera* L. *var. chinensis*.[73] 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.[74] Also, RV reduced the expression of N protein and contrasted virus-induced apoptosis in MERS-CoV-infected cells.[75] *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.[76,77] *In silico* analysis demonstrated the ability of RV and its derivatives to strongly and stably block SARS-CoV-2 proteins PL^{PRO}, RdRp, and S protein.[78,79] 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 PL^{PRO}-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.[80] 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.[81] Catechin interfered with SARS-CoV-2 infection and replication by neutralizing $3CL^{PRO}$, S protein RBD, ACE2, S/ACE2 complex, cathepsin L, nsp6, and N protein.[82–84] Tea polyphenols, including epigallocatechin-3-O-gallate (EGCG) and theaflavin 3,3'di-gallate, can strongly dock to $3CL^{PRO}$, S protein, S/ACE2 complex, PL^{PRO}, and RdRp.[85–87] Also, a computational analysis revealed that several compounds with a common catechin skeleton can inhibit SARS-CoV-2 protein N.[88] 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 aungustifolium Ait* (blueberry), *Vitis vinifera* L. (grape seed), and *Cinnamomum cassia Presl* (cinnamon bark).[89] Procyanidin can significantly inhibit IAV replication in a concentration-dependent manner with no cytotoxicity.[90] Importantly, procyanidin's antiviral mechanism is associated with reduced production and accumulation of LC3-II and decreased expression of ATG5, ATG7, and ATG12 [90]. 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

Table 1
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, TMPSS2 receptor, Cathepsin-L (CTSL)) and autophagosome and DMV biogenesis (nsp3/PL^{PRO}, nsp4, nsp6, nsp8, $3CL^{PRO}$, 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** ↑ BNIP3 ↑ MMP ↑ LC3-II ↓ p62 ↓ mtROS ↓ caspase-1 ↓ 1L-1β ↓ NLRP3 (Liu et al., 2020) **Autophagy inhibition (EV71) -**(BBR and its derivative 2d) ↑ Akt ↑ SQSTM1/p62 ↓ JNK ↓ PI3K-III ↓ LC3B-II ↓ MEK/ERK No effect on Beclin-1 (Wang et al., 2017; Wang et al., 2018)	• **SARS-Cov-2 $3CL^{PRO}$**: 83.2 kcal/mol *S protein: 69.*70 kcal/mol *ACE-2: 71.*50 kcal/mol *(Lakshmi* et al., *2020)* • ***SARS-Cov-2 $3CL^{PRO}$:*** *7.*3 kcal/mol *(Chowdhury* et al., *2020)* • ***Immunotherapeutic-Berberine nanomedicine (NIT-X)*** *MAPK3: 8.*9 kcal/mol *MAPK8: 8.*6 kcal/mol *TNF-α: 8.*2 kcal/mol*)* *MAPK1: 8.*2 kcal/mol *BAX: 8.*1 kcal/mol*)* *NF-Kβ1: 7.*3 kcal/mol *CHUK: 7.*3 kcal/mol *ACE-2 receptor: 9.*8 kcal/mol *(Wang* et al., *2020)*
Baicalin	**Autophagy inhibition (H3N2)** ↑ mTOR ↓ LC3-II/GAPDH ratio ↓ Atg5-Atg12 (Zhu et al., 2015)	• ***SARS-CoV-2 $3CL^{PRO}$:*** *Baicalein: 8.277* *Baicalin: 8.458* *Herbacetin: 9.402 (Liu* et al., *2021)* • ***SARS-CoV-2*** *and* ***2′-O-ribose methyltransferase (MTase) nps16****: 8,*7 kcal/mol *(Chandra* et al., *2021)* • ***SARS-CoV-2 $3CL^{PRO}$****: 8.*1 kcal/mol *(Islam* et al., *2020)* • ***SARS-CoV-2 $3CL^{PRO}$*** *inhibitors – best docking scores:* *Withaferin-A: 9.*22 kcal/mol *Hesperidin: 2.*87 kcal/mol *Baicalin: 6.*68 kcal/mol*)* *21 Baicalin derivatives: 5.45 to - 7.*78 kcal/mol *(Ghosh* et al., *2021)* • ***SARS-CoV-2 $3CL^{PRO}$****:* *Baicalin: 8.776* *Herbacetin: 8.738* *Pectolinarin: 10.969 (Jo* et al., *2020)* • ***SARS-CoV-2 nsp14 N-****terminal* ***and C-terminal****:* *Baicalein: 8.*8 kcal/mol *and - 8.*7 kcal/mol*, respectively. (Liu* et al., *2020)* • ***SARS-CoV-2****:* *Nsp4: 6.*8 kcal/mol *Nsp15: 7.*4 kcal/mol *RdRp: 8.*7 kcal/mol *(Alazmi and Motwalli, 2020)* • ***SARS-CoV-2****:* *S protein: 5.*56 kcal/mol *$3CL^{PRO}$: 6.*58 kcal/mol *PL^{PRO}: 5.*95 kcal/mol *Furin: 7.*40 kcal/mol *(Manikyam and Joshi, 2020)*
Resveratrol (RES) and RES-loaded nanoparticles	**Autophagy inhibition (EV71)** ↓ p-PERK, P-eIF2, ATF4, GRP78, and CHOP ↓ LC3-II ↓ LC3-II/LC3-I ratio ↓ IL-6, IL-8, and TNF-α ↓ MDA and ROS ↑ SOD (Du et al., 2019)	• ***RES and SARS-CoV-2 PL^{PRO} (6W9C) and RdRp (6M71)*** *- strongest inhibitions:* *RV-13 (PL^{PRO} - 184.99 kj/mol)* *RV12 (RdRp -173.76 kj/mol)* *(Ranjbar* et al., *2020)* • ***RES*** > **Most *stable interaction with S1/ACE-2 complex****: 8.*0 kcal/mol *(Wahedi* et al., *2020)*
Catechin (C) and Tea Polyphenols	**Autophagy inhibition (H1N1)** ↓ M2 ↓ NP ↓ LC3B ↓ PI3K-III (Chang et al., 2020)	• ***Catechin - SARS-CoV-2:*** *$3CL^{PRO}$: 8.*34 kcal/mol *CTSL: 7.*68 kcal/mol *S protein RBD: 5.*79 kcal/mol *nsp6: 7.*04 kcal/mol *N protein: 6.*23 kcal/mol*)* *(cut off range > 5.*0 kcal/mol*)* *(Mishra* et al., *2020)* • ***Catechin - SARS-****CoV****-2:*** *S protein: 10.*5 kcal/mol *ACE-2: 8.*9 kcal/mol *S-RBD/ACE-2 complex: 9.*1 kcal/mol *(Jena* et al., *2021)* • ***Tea Polyphenols – SARS-CoV-2:*** *$3CL^{PRO}$-EGCG: 8.*3 kcal/mol *$3CL^{PRO}$-TF3: 8.*4 kcal/mol *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)*
		*S RBD-TF3: 11.*6 kcal/mol *PL^{PRO}-EGCG: 8.*9 kcal/mol *PL^{PRO}-TF3: 11.*3 kcal/mol *RdRp-EGCG: 5.*7 kcal/mol *RdRp-TF3:* 6.0 kcal/mol *ACE2/S RBD-EGCG: 8.*5 kcal/mol *ACE2/S RBD-TF3: 8.*0 kcal/mol *(Mhatre* et al., *2021)*
Procyanidins	**Autophagy inhibition (IAV)** ↓ LC3-II/β-actin ratio ↓ LC3-II accumulation ↓ LCB3-I to II conversion ↓ Atg7, 5, and 12 expression ↓ Atg5-Atg12/Atg16 heterotrimer Stabilized beclin1/bcl2 heterodimer (Dai et al., 2012–1)	• ***Proanthocyanidins and Catechins - SARS-CoV-2 $3CL^{PRO}$:*** *Procyanidin B2 (PB2): 9.*2 kcal/mol *Procyanidin A2 (PA2): 9.2* *(−)-epigallocatechin-3-O-gallate (EGCG): 8.7* *(−)-gallocatechin-3-O-gallate (GCG): 8.7* *(−)-epicatechin-3-O-gallete (ECG): 8.7* (+)-catechin-3-O-gallate (CAG): −8.3 (−)-epigallocatechin (EGC): 7.7 *(+)-gallocatechin (GC): 7.6* *(−)-epicatechin (EPC): 7.5* *(+)-catechin (CA): 7.5* *(−)-epiafzelechin (EAF): 7.5* *(−)-afzelechin (AF): 7.0* *Lopinavir (LOP): 8.0* *Ebselen (EBS): 6.6* *Cinanserin (INN): 5.4)* *(Zhu and Xie, 2020)* • *Procyanidins**, Theaflavin, and Theasinensins**:* *RdRp:* *Theaflavin 3,3'di-gallate (TF3): 14.92* *Theaflavin 3′-gallate (TF2a): 13.26* *Digalloylprocyanidin B2: 13.26* *$3CL^{PRO}$:* *Procyanidin: 11.68* *TF2a: 11.52* *Theaflavin: 11.07* *PL^{PRO}:* *TF2a: 10.90* *Theasinensin A: 10.81* *Theasinensin B: 10.73 (Gogoi* et al., *2021)* • ***Procyanidins**:* *Nsp1: 8.*5 kcal/mol *Nsp2: 8.*8 kcal/mol *PL^{PRO}: 9.*7 kcal/mol *Nsp4: 9.*7 kcal/mol *Nsp6: 8.*9 kcal/mol *Nsp7: 8.*1 kcal/mol *Nsp8: 8.*7 kcal/mol *Nsp9: 8.*4 kcal/mol *Nsp10: 8.*3 kcal/mol *RdRp: 8.*9 kcal/mol *Helicase: 9.*5 kcal/mol *ExonN: 9.*6 kcal/mol *NendoU: 8.*2 kcal/mol *2′-O-MT: 10.*1 kcal/mol *ORF3a: 7.*9 kcal/mol *E protein: 9.*0 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: 9.*7 kcal/mol *ORF10: 7.*2 kcal/mol *ACE-2: 8.*9 kcal/mol *M^{PRO}: 9.*2 kcal/mol *S protein: 9.*5 kcal/mol *(Maroli* et al., *2020)*
Quercetin-7-0-glucoside (Q7G)	**Autophagy inhibition (IAV) – *in vitro*** ↓ Acidic Vesicular Organelles (AVO) ↓ Atg-5, Atg-7 ↓ LCB-3 ↓ ROS (Gansukh et al., 2016)	• *Q7G blocks the PB2 subunit of the RdRp: 9.*5 kcal/mol *(***Gansukh** et al., *2016)* • *SARS-CoV-2 $3CL^{PRO}$: 6.*25 kcal/mol *PL^{PRO}: 4.*62 kcal/mol *(Zhang* et al., *2020)* • *$3CL^{PRO}$: 7.5 to -7.*2 kcal/mol *(Abian* et al., *2020)<* • *S protein: 8.*5 kcal/mol *(Pandey* et al., *2020)* • *$3CL^{PRO}$: 6.*58 kcal/mol *Nsp15: 6.*49 kcal/mol *(Sharma* et al., *2020)* • *$3CL^{PRO}$: 6.*71 kcal/mol *S protein RBD/ACE-s complex: 5.*56 kcal/mol *S protein: 5.*19 kcal/mol

(*continued on next page*)

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)*
		*RdRp: 5.*89 kcal/mol *PL^{PRO}: 5.*41 kcal/mol *Others: see original paper (Saakre* et al., *2021)*
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 ↑ LC3-II accumulation ↑ p62 No effect on mTOR SsD-mediated autophagy > independent of ER or lysosomal Ca^{2+} pools (Li et al., 2019)	• *Saikosaponins **and SARS-CoV-2**:* *Nsp15: 8.358 to -* 3.738 kcal/mol *S protein: 8.299 to -5.638* *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-SsB4: 7.*981 kcal/mol *JAK3-SsI: 7.*942 kcal/mol *NOX5-SsBK1: 7.*813 kcal/mol *NOX5-SsC: 9.*202 kcal/mol *Best interactions: JAK3-Ss compounds* *Saikosaponins interacted with CAT Gene CAT (Catalase) and Checkpoint kinase (CHEK1)* *(Chikhale* et al., *2021)*
Eugenol	**Autophagy inhibition (IAV) – *in vitro*** ↓ ERK ↓ p38 MAPK ↓ IKK/NF-Kβ ↓ dissociation of Beclin 1-Blc-2 heterodimer ↓ TNF-α, IL-6, and IL-8 (Dai et al., 2013)	• *Eugenol:* *SARS-CoV-2 3CL^{PRO} (6LU7): 5.*4 kcal/mol *S protein (6VXX): 6.*1 kcal/mol *EGCG:* *SARS-CoV-2 S protein: 9.*8 kcal/mol *3CL^{PRO}: 7.*8 kcal/mol kcal/mol *Hesperidin:* *SARS-CoV-2 3CL^{PRO} (6LU7): 8.*3 kcal/mol *S protein (6VXX): 10.*4 kcal/mol *(Tallei* et al., *2020)* • ***Eugenol** – **SARS-CoV-2:*** *SARS-CoV-2 3CL^{PRO}: 3.*7 kcal/mol Nsp15: 3.8 kcal/mol RdRp: 3.2 kcal/mol *(Saxena* et al., *2021)* • ***Eugenol** – **SARS-CoV-2:*** *SARS-CoV-2 3CL^{PRO}: 93.2 kj/mol* *Nsp15: 91.7 kj/mol* *ADP ribose phosphatase: 105.2 kj/mol* *RdRp: 80.0 kj/mol* *S protein: 79.1 kj/mol* *ACE-2: 88.4 kj/mol (*≤ 80 kJ/mol*: strongest docking activity)* *(Da Silva* et al., *2020)*
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^{PRO}:*** *PGG: 6.*4 kcal/mol *EGCG: H41 residue - 3.*5 kcal/mol *and C145 residue - 6.*0 kcal/mol *(Chiou* et al., *2021)* • ***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® 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 Amantadine-M2 (control): −4.52 kcal/mol (Choi et al., 2019)	***Aloe vera molecules** - SARS-CoV-2 3CL^{PRO}:* *Feralolide: 7.*9 kcal/mol *9-dihydroxyl-2-O-(z)-cinnamoyl-7-methoxy-aloesia: 7.*7 kcal/mol *Aloeresin: 7.*7 kcal/mol *Isoaloeresin: 7.*3 kcal/mol *Aloin A: 7.*1 kcal/mol *Elgonica dimer A: 7.*1 kcal/mol *7-O-methylaloeresin: 7.*0 kcal/mol *Chrysophanol: 6.*8 kcal/mol *Aloe-emodin: 6.*7 kcal/mol *Aloin B: 6.*7 kcal/mol *(Mpiana* et al., *2020)*
Evodiamine	**Autophagy inhibition (IAV) – *in vitro*** ↓ Atg5-Atg12/Atg16 heterotrimer formation ↓ Atg5, Atg12, and Atg16 protein expression ↓ LC3-II and p62 accumulation ↓ AMPK/↓ 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/β-actin) ↓ LC3B–I to LC3B-II conversion ↑ p62 ↓ Beclin-1 (Liao et al., 2020)	*No articles found.*
Kurarinone		*No articles found.*

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)*
	Autophagy inhibition (HCoV-OC43) – *in vitro* ↓ LC3-I/LC3-II transversion (LC3-II/LC3-I ratio) ↑ p62/SQSTM1 (Min et al., 2020)	

Table 2
Summary of herbs-compounds that may potentially inhibit SARS-CoV-2/host proteins. The proteins/receptors that would be especially important to prevent or inhibit SARS-CoV-2-induced autophagy are S, ACE-2, S/ACE-2 complex, TMPSS2, Furin, CTSL, nsp2, nsp3/PL^{PRO}, nsp4, nsp5/$3CL^{PRO}$, nsp6, and nsp8. This table shows the herbal-protein interactions. For specific docking scores see Table 1.

SARS-CoV-2/host proteins	Herbs/Compounds
S protein	*Berberine, Baicalin, Saikosaponins, Catechin (C), EGCG, TF3, Procyanidins, Eugenol, and Quercetins*
ACE-2 receptor	*Berberine, immunotherapeutic-BBR nanomedicine (NIT-X), Catechin (C), Procyanidins, Eugenol*
S/ACE-2 complex	*Resveratrol, Catechin (C), EGCG, TF3, Quercetins, PGG*
Furin	*Baicalin*
TMPSS2 receptor	*NIT-X*
Cathepsin-L (CTSL)	*Catechin (C)*
Nsp1	*Procyanidins*
Nsp2	*Procyanidins*
PL^{PRO} (is a domain of nsp3)	*Baicalin (interacted with PL^{PRO}), Resveratrol, EGCG, TF3, TF2a, Theasinesin, Procyanidins, Quercetins*
ADP ribose phosphatase	*Eugenol*
Nsp4	*Baicalin, Procyanidins*
Nsp5/$3CL^{PRO}$ (processes nsp4-16)	*Berberine, Scutellaria baicalensis, baicalein, baicalin, baicalin derivatives, Catechin (C), EGCG, TF3, Procyanidins, GCG, ECG, CAG, EGC, GC, EPC, EAF, AF, TF2a, Eugenol, PGG, Quercetins, Aloe vera compounds (e.g. feralolide, 9-dihydroxyl-2-O-(z)-cinnamoyl-7-methoxy-aloesia and aloeresin)*
Nsp6	*Catechin (C), Procyanidins*
Nsp7	*Procyanidins*
Nsp8	*Procyanidins*
Nsp9	*Procyanidins*
Nsp10	*Procyanidins*
Nsp12 (RdRp)	*Baicalin, Resveratrol, EGCG, TF3, TF2a, Procyanidins, Eugenol, Quercetins*
Nsp13 (Helicase)	*Procyanidins*
Nsp14 (ExonN)	*Baicalein (nsp14 N and C terminals), Procyanidins*
Nsp15 (NendoU)	*Baicalin, Saikosaponins, Procyanidins, Eugenol, Quercetins*
***2′-O-ribose methyltransferase* (MTase) - nsp16**	*Baicalin, Procyanidins*
N protein	*Catechin (C), catechin skeleton compounds, Procyanidins*
ORF3a, E protein, M protein, ORF6, ORF7a, ORF8, ORF10	*Procyanidins*

capacity of procyanidins to significantly interact with SARS-CoV-2 $3CL^{PRO}$, nsp1, nsp2, PL^{PRO}, nsp4, nsp6, nsp7, nsp8, nsp9, nsp10, RdRp, helicase, exon N, NendoU, 2′-O-MT, ORF3a, E protein, M protein, ORF6, ORF7a, ORF8, N protein, ORF10, ACE2, and S protein.[84,87,92] Interestingly, flavan-3-ols and procyanidins were also associated with ACE inhibition *in vitro*.[93] These studies highlight the extension of the potential of flavanols (e.g. procyanidins and catechins) to inhibit SARS-CoV-2/host proteins, especially those involved in DMV biogenesis and the regulation of autophagy. However, like catechins, procyanidins have a limited absorption,[94] and modes of administration that optimize cell delivery may need to be developed to further the SARS-CoV-2 model studies.

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 IAV replication by blocking viral RNA synthesis, and in parallel prevented the accumulation of acidic vesicular organelles.[95] However, the labeling of acidic organelles with acridine orange indicates that Q7G only prevented the organelle acidification during the viral infection, and nothing can be said about autophagy. The molecular docking analysis revealed that Q7G blocks the PB2 subunit of the RdRp (- 9.5 kcal/mol), which is important for RNA viral synthesis.[95] *In silico* studies have evidenced different interaction strengths of quercetin(s) with SARS-CoV-2 $3CL^{PRO}$, S protein, S/ACE2 complex, RdRp, PL^{PRO}, and nsp15.[96–100] Based on the above, it is conceivable that quercetins could prevent SARS-CoV-2 endocytosis, DMV formation, and SARS-CoV-2-dysregulation of autophagy (the latter, by blocking PL^{PRO} and $3CL^{PRO}$-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-O-galloyl-β-D-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.[101] This was interpreted as autophagy inhibition rather than stimulation of autophagy in the early stages.[101] Also, saikosaponins (A and B2) have shown anti-human coronavirus 229E (hCoV-229E) activity *in vitro* with no toxicity.[102] SsB_2 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.[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.[101] *In silico* studies have ascertained the capacity of saikosaponins to potently block SARS-CoV-2 S protein and nsp15.[103] 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 *Sygygium aromaticum*, inhibited IAV replication along with the inhibition of autophagy.[104] One possible mechanism for downregulation of autophagy relies on the ability of Eugenol to inhibit the dissociation of BECLIN1-BCL-2 heterodimer.[104] 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 $3CL^{PRO}$, 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.[86,105,106]

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.[107] On the contrary, PGG was active against IAV, limiting the expression of viral M2 and NP proteins through induction of autophagy.[108] *In silico* studies show that PGG can interact with SARS-CoV-2 $3CL^{PRO}$ and S/ACE2 complex[109,110] (Supplementary Table 1). Additional *in vitro* and FRET analyses also demonstrated that PGG may hinder SARS-CoV-1 and SARS-CoV-2 [109]. 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.[111] *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.[111] 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.[111] Based on the *in silico* activity against $3CL^{PRO}$, *Aloe vera* molecules may indirectly inhibit DMV formation and SARS-CoV-2 induced autophagy[112] (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.[113] 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 trifoliate* (root).[114,115] 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.[115] 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.[115] Whether deguelin can exert similar antiviral activity on CoVs has not been investigated yet.

Kurarinone, a flavanone present in *S. flavescens*, has demonstrated its ability to inhibit HCoV-OC43 *in vitro* in a dose-dependent manner (IC_{50} 3.34 μM) at the early stage of infection.[116] This antiviral activity was paralleled by inhibition of autophagy, as indicated by decreased LC3-I/LC3-II conversion and increased level of p62/SQSTM1.[116] 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., its modality 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.[15] 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 *chinkuna* 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, cytotoxicity, 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.[61,74] Since viruses need to import or synthesize lipid constituents to make DMVs,[62] 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 in[117]). Viral mutations can be neutral, beneficial, or deleterious. So far, the majority of mutations

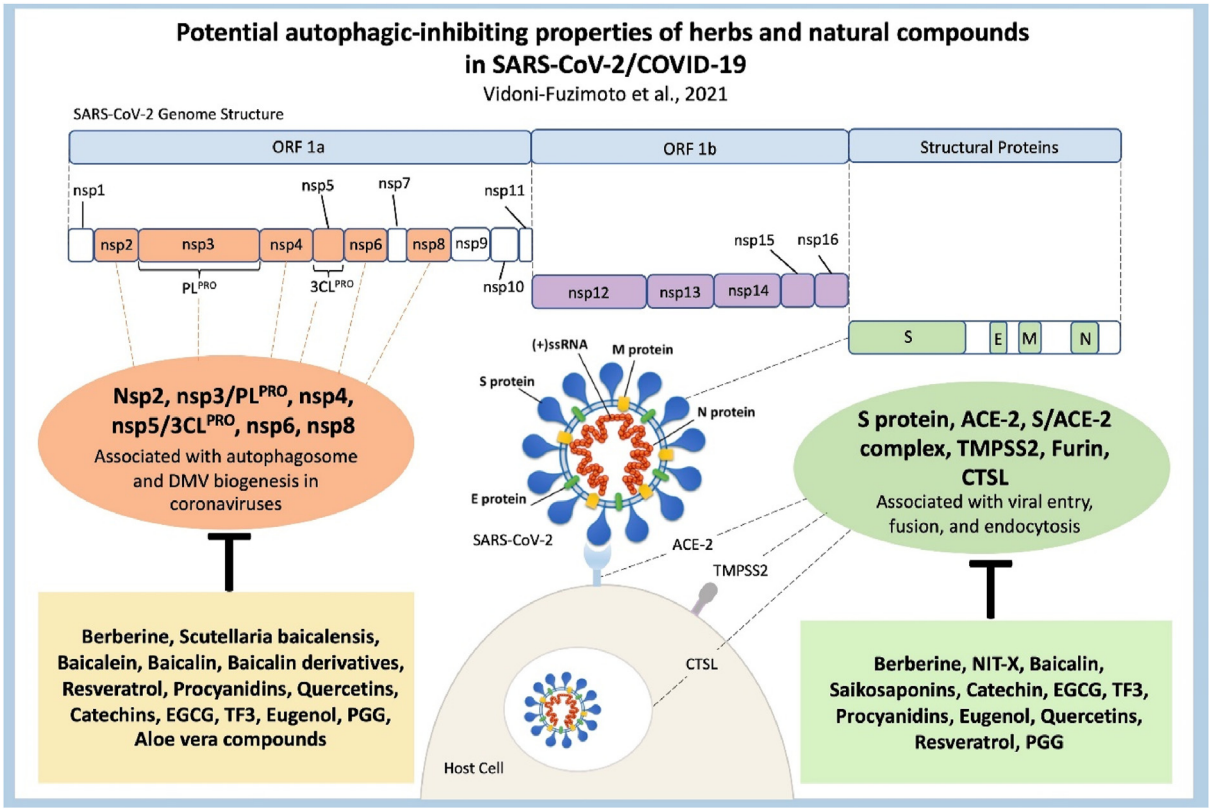

Fig. 3. Potential autophagic-inhibiting properties of herbs and natural compounds in SARS-CoV-2/COVID-19. The *in silico* research on herbs-compounds that may target SARS-CoV-2 proteins involved in autophagy and vesicle-dependent viral entry and replication. For all docking scores, see Table 1.

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.[12,121] 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 (3CLPRO, E protein, helicase, N protein, and nsp3, 10, 14, and 15), artemisinin (3CLPRO, 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, antipyretic, 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

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 AFe: literature searching and drafting of chapters 1–3, drawing of Figs. 1 and 2 and of Graphical Abstract; AFu: literature search and drafting of chapters 4, limitations and perspectives, conclusion, Tables 1 and 2, and Fig. 3; CI: conceptualization, coordination of the teamwork, revision, harmonization, and finalization of the whole manuscript. All authors have read and approved the last version.

Declaration of competing interest

Nothing to declare.

Acknowledgements

CV is supported with a post-doctoral fellowship from Università del Piemonte Orientale (Novara, Italy) in collaboration with Università degli Studi Magna Græcia (Catanzaro, Italy). AFe 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.jtcme.2021.10.003.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
2. Quiles JL, Rivas-García L, Varela-López A, Llopis J, Battino M, Sánchez-González C. Do nutrients and other bioactive molecules from foods have anything to say in the treatment against COVID-19? *Environ Res*. 2020;191:110053. https://doi.org/10.1016/j.envres.2020.110053.
3. Islam MT, Sarkar C, El-Kersh DM, et al. Natural products and their derivatives against coronavirus: a review of the non-clinical and pre-clinical data. *Phytother Res*. 2020;34(10):2471–2492. https://doi.org/10.1002/ptr.6700.
4. Colunga Biancatelli RML, Berrill M, Catravas JD, Marik PE. Quercetin and vitamin C: an experimental, synergistic therapy for the prevention and treatment of SARS-CoV-2 related disease (COVID-19). *Front Immunol*. 2020;11:1451. https://doi.org/10.3389/fimmu.2020.01451.
5. Merarchi M, Dudha N, Das BC, Garg M. Natural products and phytochemicals as potential anti-SARS-CoV-2 drugs. *Phytother Res*. 2021. https://doi.org/10.1002/ptr.7151.
6. Alam S, Sarker MMR, Afrin S, et al. Traditional herbal medicines, bioactive metabolites, and plant products against COVID-19: update on clinical trials and mechanism of actions. *Front Pharmacol*. 2021;12:671498. https://doi.org/10.3389/fphar.2021.671498.
7. Fuzimoto AD, Isidoro C. The antiviral and coronavirus-host protein pathways inhibiting properties of herbs and natural compounds - additional weapons in the fight against the COVID-19 pandemic? *J Tradit Complement Med*. 2020;10(4):405–419. https://doi.org/10.1016/j.jtcme.2020.05.003.
8. Swargiary A, Mahmud S, Saleh MA. Screening of phytochemicals as potent inhibitor of 3-chymotrypsin and papain-like proteases of SARS-CoV2: an in silico approach to combat COVID-19. *J Biomol Struct Dyn*. 2020:1–15. https://doi.org/10.1080/07391102.2020.1835729.
9. Basu A, Sarkar A, Maulik U. Molecular docking study of potential phytochemicals and their effects on the complex of SARS-CoV2 spike protein and human ACE2. *Sci Rep*. 2020;10(1):17699. https://doi.org/10.1038/s41598-020-74715-4.
10. Limanaqi F, Busceti CL, Biagioni F, et al. Cell clearing systems as targets of polyphenols in viral infections: potential implications for COVID-19 pathogenesis. *Antioxidants*. 2020;9(11):1105. https://doi.org/10.3390/antiox9111105.
11. Miller K, McGrath ME, Hu Z, et al. Coronavirus interactions with the cellular autophagy machinery. *Autophagy*. 2020;16(12):2131–2139. https://doi.org/10.1080/15548627.2020.1817280.
12. Sargazi S, Sheervalilou R, Rokni M, Shirvaliloo M, Shahraki O, Rezaei N. The role of autophagy in controlling SARS-CoV-2 infection: an overview on virophagy-mediated molecular drug targets. *Cell Biol Int*. 2021;45(8):1599–1612. https://doi.org/10.1002/cbin.11609.
13. Sumpter Jr R, Levine B. Selective autophagy and viruses. *Autophagy*. 2011;7(3):260–265. https://doi.org/10.4161/auto.7.3.14281.
14. Yin Z, Pascual C, Klionsky DJ. Autophagy: machinery and regulation. *Microb Cell*. 2016;3(12):588–596. https://doi.org/10.15698/mic2016.12.546.
15. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). *Autophagy*. 2021;17(1):1–382. https://doi.org/10.1080/15548627.2020.1797280.
16. Corona Velazquez AF, Jackson WT. So many roads: the multifaceted regulation of autophagy induction. *Mol Cell Biol*. 2018;38(21):e00303–e00318. https://doi.org/10.1128/MCB.00303-18.
17. Codogno P, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol*. 2011;13(1):7–12. https://doi.org/10.1038/nrm3249.
18. Dupont N, Codogno P. Non-canonical autophagy: facts and prospects. *Curr Pathobiol Rep*. 2013;1:263–271. https://doi.org/10.1007/s40139-013-0030-y.
19. Al-Bari MAA, Xu P. Molecular regulation of autophagy machinery by mTOR-dependent and -independent pathways. *Ann N Y Acad Sci*. 2020;1467(1):3–20. https://doi.org/10.1111/nyas.14305.
20. Wang J, Whiteman MW, Lian H, et al. A non-canonical MEK/ERK signaling pathway regulates autophagy via regulating Beclin 1. *J Biol Chem*. 2009;284(32):21412–21424. https://doi.org/10.1074/jbc.M109.026013.
21. Zhou YY, Li Y, Jiang WQ, Zhou LF. MAPK/JNK signalling: a potential autophagy regulation pathway. *Biosci Rep*. 2015;35(3), e00199. https://doi.org/10.1042/BSR20140141.
22. Sanjuan MA, Dillon CP, Tait SW, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature*. 2007;450(7173):1253–1257. https://doi.org/10.1038/nature06421.
23. Sil P, Muse G, Martinez J. A ravenous defense: canonical and non-canonical autophagy in immunity. *Curr Opin Immunol*. 2018;50:21–31. https://doi.org/10.1016/j.coi.2017.10.004.
24. Pleet ML, Branscome H, DeMarino C, et al. Autophagy, EVs, and infections: a perfect question for a perfect time. *Front Cell Infect Microbiol*. 2018;8:362. https://doi.org/10.3389/fcimb.2018.00362.
25. Wang Y, Sharma P, Jefferson M, et al. Non-canonical autophagy functions of ATG16L1 in epithelial cells limit lethal infection by influenza A virus. *EMBO J*. 2021;40(6), e105543. https://doi.org/10.15252/embj.2020105543. Epub 2021 Feb 15.
26. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol*. 2015;1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.
27. Chan JF, Kok KH, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microb Infect*. 2020;9(1):221–236. https://doi.org/10.1080/22221751.2020.1719902.
28. Wong NA, Saier Jr MH. The SARS-coronavirus infection cycle: a survey of viral membrane proteins, their functional interactions and pathogenesis. *Int J Mol Sci*. 2021;22(3):1308. https://doi.org/10.3390/ijms22031308.
29. Burkard C, Verheije MH, Wicht O, et al. Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner. *PLoS Pathog*. 2014;10(11), e1004502. https://doi.org/10.1371/journal.ppat.1004502.
30. Tatematsu M, Funami K, Seya T, Matsumoto M. Extracellular RNA sensing by pattern recognition receptors. *J Innate Immun*. 2018;10(5-6):398–406. https://doi.org/10.1159/000494034.
31. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun*. 2020;11(1):1620. https://doi.org/10.1038/s41467-020-15562-9.
32. Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antivir Res*. 2020;178:104792. https://doi.org/10.1016/j.antiviral.2020.104792.
33. Angelini MM, Akhlaghpour M, Neuman BW, Buchmeier MJ. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *mBio*. 2013;4(4). https://doi.org/10.1128/mBio.00524-13. e00524-13.
34. Ghosh S, Dellibovi-Ragheb TA, Kerviel A, et al. β-Coronaviruses use lysosomes for egress instead of the biosynthetic secretory pathway. *Cell*. 2020;183(6):1520–1535. https://doi.org/10.1016/j.cell.2020.10.039. e14.
35. Jackson WT. Viruses and the autophagy pathway. *Virology*. 2015;479–480:450–456. https://doi.org/10.1016/j.virol.2015.03.042.
36. Reggiori F, Monastyrska I, Verheije MH, et al. Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell Host Microbe*. 2010;7(6):500–508. https://doi.org/10.1016/j.chom.2010.05.013.
37. Bello-Perez M, Sola I, Novoa B, Klionsky DJ, Falco A. Canonical and noncanonical autophagy as potential targets for COVID-19. *Cells*. 2020;9(7):1619. https://doi.org/10.3390/cells9071619.
38. Zhao Z, Thackray LB, Miller BC, et al. Coronavirus replication does not require

the autophagy gene ATG5. *Autophagy*. 2007;3(6):581–585. https://doi.org/10.4161/auto.4782.
39. Choi Y, Bowman JW, Jung JU. Autophagy during viral infection - a double-edged sword. *Nat Rev Microbiol*. 2018;16(6):341–354. https://doi.org/10.1038/s41579-018-0003-6.
40. Cottam EM, Maier HJ, Manifava M, et al. Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. *Autophagy*. 2011;7(11):1335–1347. https://doi.org/10.4161/auto.7.11.16642.
41. Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. *Autophagy*. 2014;10(8):1426–1441. https://doi.org/10.4161/auto.29309.
42. Jackson WT, Giddings Jr TH, Taylor MP, et al. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol*. 2005;3(5):e156. https://doi.org/10.1371/journal.pbio.0030156.
43. Chen X, Wang K, Xing Y, et al. Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. *Protein Cell*. 2014;5(12):912–927. https://doi.org/10.1007/s13238-014-0104-6.
44. Gassen NC, Niemeyer D, Muth D, et al. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. *Nat Commun*. 2019;10(1):5770. https://doi.org/10.1038/s41467-019-13659-4.
45. Kuballa P, Nolte WM, Castoreno AB, Xavier RJ. Autophagy and the immune system. *Annu Rev Immunol*. 2012;30:611–646. https://doi.org/10.1146/annurev-immunol-020711-074948.
46. Münz C. Autophagy proteins in antigen processing for presentation on MHC molecules. *Immunol Rev*. 2016;272(1):17–27. https://doi.org/10.1111/imr.12422.
47. Oh DS, Park JH, Jung HE, Kim HJ, Lee HK. Autophagic protein ATG5 controls antiviral immunity via glycolytic reprogramming of dendritic cells against respiratory syncytial virus infection. *Autophagy*. 2020:1–17. https://doi.org/10.1080/15548627.2020.1812218. Epub ahead of print.
48. Yang CS, Rodgers M, Min CK, et al. The autophagy regulator Rubicon is a feedback inhibitor of CARD9-mediated host innate immunity. *Cell Host Microbe*. 2012;11(3):277–289. https://doi.org/10.1016/j.chom.2012.01.019.
49. Warowicka A, Nawrot R, Goździcka-Józefiak A. Antiviral activity of berberine. *Arch Virol*. 2020;165(9):1935–1945. https://doi.org/10.1007/s00705-020-04706-3. Epub 2020 Jun 28.
50. Ding Y, Dou J, Teng Z, et al. Antiviral activity of baicalin against influenza A (H1N1/H3N2) virus in cell culture and in mice and its inhibition of neuraminidase. *Arch Virol*. 2014;159(12):3269–3278. https://doi.org/10.1007/s00705-014-2192-2. Epub 2014 Jul 31.
51. Shi H, Ren K, Lv B, et al. Baicalin from Scutellaria baicalensis blocks respiratory syncytial virus (RSV) infection and reduces inflammatory cell infiltration and lung injury in mice. *Sci Rep*. 2016;6:35851. https://doi.org/10.1038/srep35851.
52. Oo A, Rausalu K, Merits A, et al. Deciphering the potential of baicalin as an antiviral agent for Chikungunya virus infection. *Antivir Res*. 2018;150:101–111. https://doi.org/10.1016/j.antiviral.2017.12.012. Epub 2017 Dec 19.
53. Li BQ, Fu T, Dongyan Y, Mikovits JA, Ruscetti FW, Wang JM. Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. *Biochem Biophys Res Commun*. 2000;276(2):534–538. https://doi.org/10.1006/bbrc.2000.3485.
54. Moghaddam E, Teoh BT, Sam SS, et al. Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. *Sci Rep*. 2014;4:5452. https://doi.org/10.1038/srep05452.
55. Abba Y, Hassim H, Hamzah H, Noordin MM. Antiviral activity of resveratrol against human and animal viruses. *Adv Virol*. 2015;2015:184241. https://doi.org/10.1155/2015/184241. Epub 2015 Nov 29.
56. Neag MA, Mocan A, Echeverría J, et al. Berberine: botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. *Front Pharmacol*. 2018;9:557. https://doi.org/10.3389/fphar.2018.00557.
57. Liu H, You L, Wu J, et al. Berberine suppresses influenza virus-triggered NLRP3 inflammasome activation in macrophages by inducing mitophagy and decreasing mitochondrial ROS. *J Leukoc Biol*. 2020;108(1):253–266. https://doi.org/10.1002/JLB.3MA0320-358RR.
58. Wang YX, Yang L, Wang HQ, et al. Synthesis and evolution of berberine derivatives as a new class of antiviral agents against enterovirus 71 through the MEK/ERK pathway and autophagy. *Molecules*. 2018;23(8):2084. https://doi.org/10.3390/molecules23082084.
59. Alagu Lakshmi S, Shafreen RMB, Priya A, Shunmugiah KP. Ethnomedicines of Indian origin for combating COVID-19 infection by hampering the viral replication: using structure-based drug discovery approach. *J Biomol Struct Dyn*. 2020:1–16. https://doi.org/10.1080/07391102.2020.1778537.
60. Chowdhury P. In silico investigation of phytoconstituents from Indian medicinal herb 'Tinospora cordifolia (giloy)' against SARS-CoV-2 (COVID-19) by molecular dynamics approach. *J Biomol Struct Dyn*. 2020:1–18. https://doi.org/10.1080/07391102.2020.1803968.
61. Wang ZZ, Li K, Maskey AR, et al. A small molecule compound berberine as an orally active therapeutic candidate against COVID-19 and SARS: a computational and mechanistic study. *Faseb J*. 2021;35(4), e21360. https://doi.org/10.1096/fj.202001792R.
62. Wolff G, Melia CE, Snijder EJ, Bárcena M. Double-membrane vesicles as platforms for viral replication. *Trends Microbiol*. 2020;28(12):1022–1033. https://doi.org/10.1016/j.tim.2020.05.009.
63. Varghese FS, van Woudenbergh E, Overheul GJ, et al. Berberine and obatoclax inhibit SARS-cov-2 replication in primary human nasal epithelial cells in vitro. *Viruses*. 2021;13(2):282. https://doi.org/10.3390/v13020282.
64. Pizzorno A, Padey B, Dubois J, et al. In vitro evaluation of antiviral activity of single and combined repurposable drugs against SARS-CoV-2. *Antivir Res*. 2020;181:104878. https://doi.org/10.1016/j.antiviral.2020.104878.
65. Batista MN, Braga ACS, Campos GRF, et al. Natural products isolated from oriental medicinal herbs inactivate zika virus. *Viruses*. 2019;11(1):49. https://doi.org/10.3390/v11010049.
66. Zhu HY, Han L, Shi XL, et al. Baicalin inhibits autophagy induced by influenza A virus H3N2. *Antivir Res*. 2015;113:62–70. https://doi.org/10.1016/j.antiviral.2014.11.003.
67. Chandra A, Chaudhary M, Qamar I, Singh N, Nain V. In silico identification and validation of natural antiviral compounds as potential inhibitors of SARS-CoV-2 methyltransferase. *J Biomol Struct Dyn*. 2021:1–11. https://doi.org/10.1080/07391102.2021.1886174.
68. Manikyam HK, Joshi SK. Whole genome analysis and targeted drug discovery using computational methods and high throughput screening tools for emerged novel coronavirus (2019-nCoV). *J Pharm Drug Res*. 2020;3(2):341–361. PMID: 32617527; PMCID: PMC7331973.
69. Liu H, Ye F, Sun Q, et al. Scutellaria baicalensis extract and baicalein inhibit replication of SARS-CoV-2 and its 3C-like protease in vitro. *J Enzym Inhib Med Chem*. 2021;36(1):497–503. https://doi.org/10.1080/14756366.2021.1873977.
70. Jo S, Kim S, Kim DY, Kim MS, Shin DH. Flavonoids with inhibitory activity against SARS-CoV-2 3CLpro. *J Enzym Inhib Med Chem*. 2020;35(1):1539–1544. https://doi.org/10.1080/14756366.2020.1801672.
71. Alazmi M, Motwalli O. In silico virtual screening, characterization, docking and molecular dynamics studies of crucial SARS-CoV-2 proteins. *J Biomol Struct Dyn*. 2020:1–11. https://doi.org/10.1080/07391102.2020.1803965.
72. Liu C, Zhu X, Lu Y, Zhang X, Jia X, Yang T. Potential treatment with Chinese and Western medicine targeting NSP14 of SARS-CoV-2. *J Pharm Anal*. 2021;11(3):272–277. https://doi.org/10.1016/j.jpha.2020.08.002.
73. Tian B, Liu J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. *J Sci Food Agric*. 2020;100(4):1392–1404. https://doi.org/10.1002/jsfa.10152.
74. Du N, Li XH, Bao WG, Wang B, Xu G, Wang F. Resveratrol-loaded nanoparticles inhibit enterovirus 71 replication through the oxidative stress-mediated ERS/autophagy pathway. *Int J Mol Med*. 2019;44(2):737–749. https://doi.org/10.3892/ijmm.2019.4211.
75. Lin SC, Ho CT, Chuo WH, Li S, Wang TT, Lin CC. Effective inhibition of MERS-CoV infection by resveratrol. *BMC Infect Dis*. 2017;17(1):144. https://doi.org/10.1186/s12879-017-2253-8.
76. Pasquereau S, Nehme Z, Haidar Ahmad S, et al. Resveratrol inhibits HCoV-229E and SARS-CoV-2 coronavirus replication in vitro. *Viruses*. 2021;13(2):354. https://doi.org/10.3390/v13020354.
77. Yang M, Wei J, Huang T, et al. Resveratrol inhibits the replication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in cultured Vero cells. *Phytother Res*. 2021;35(3):1127–1129. https://doi.org/10.1002/ptr.6916.
78. Ranjbar A, Jamshidi M, Torabi S. Molecular modelling of the antiviral action of Resveratrol derivatives against the activity of two novel SARS CoV-2 and 2019-nCoV receptors. *Eur Rev Med Pharmacol Sci*. 2020;24(14):7834–7844. https://doi.org/10.26355/eurrev_202007_22288.
79. Wahedi HM, Ahmad S, Abbasi SW. Stilbene-based natural compounds as promising drug candidates against COVID-19. *J Biomol Struct Dyn*. 2021;39(9):3225–3234. https://doi.org/10.1080/07391102.2020.1762743.
80. Xu J, Xu Z, Zheng W. A review of the antiviral role of green tea catechins. *Molecules*. 2017;22(8):1337. https://doi.org/10.3390/molecules22081337.
81. Chang CC, You HL, Huang ST. Catechin inhibiting the H1N1 influenza virus associated with the regulation of autophagy. *J Chin Med Assoc*. 2020;83(4):386–393. https://doi.org/10.1097/JCMA.0000000000000289.
82. Mishra CB, Pandey P, Sharma RD, et al. Identifying the natural polyphenol catechin as a multi-targeted agent against SARS-CoV-2 for the plausible therapy of COVID-19: an integrated computational approach. *Briefings Bioinf*. 2021;22(2):1346–1360. https://doi.org/10.1093/bib/bbaa378.
83. Jena AB, Kanungo N, Nayak V, Gbn Chainy, Dandapat J. Catechin and curcumin interact with S protein of SARS-CoV2 and ACE2 of human cell membrane: insights from computational studies. *Sci Rep*. 2021;11(1):2043. https://doi.org/10.1038/s41598-021-81462-7.
84. Zhu Y, Xie DY. Docking characterization and in vitro inhibitory activity of flavan-3-ols and dimeric proanthocyanidins against the main protease activity of SARS-cov-2. *Front Plant Sci*. 2020;11:601316. https://doi.org/10.3389/fpls.2020.601316.
85. Mhatre S, Naik S, Patravale V. A molecular docking study of EGCG and theaflavin digallate with the druggable targets of SARS-CoV-2. *Comput Biol Med*. 2021;129:104137. https://doi.org/10.1016/j.compbiomed.2020.104137.
86. Tallei TE, Tumilaar SG, Niode NJ, et al. Potential of plant bioactive compounds as SARS-CoV-2 main protease (mpro) and spike (S) glycoprotein inhibitors: a molecular docking study. *Sci Tech Rep*. 2020;2020:6307457. https://doi.org/10.1155/2020/6307457.
87. Gogoi M, Borkotoky M, Borchetia S, Chowdhury P, Mahanta S, Barooah AK. Black tea bioactives as inhibitors of multiple targets of SARS-CoV-2 (3CLpro, PLpro and RdRp): a virtual screening and molecular dynamic simulation study. *J Biomol Struct Dyn*. 2021:1–24. https://doi.org/10.1080/07391102.2021.1897679.

88. Fernández JF, Lavecchia MJ. Small molecule stabilization of non-native protein-protein interactions of SARS-CoV-2 N protein as a mechanism of action against COVID-19. *J Biomol Struct Dyn*. 2020:1–8. https://doi.org/10.1080/07391102.2020.1860828.
89. Woo-Sik Jeong & Ah-Ng Tony Kong. Biological properties of monomeric and polymeric catechins: green tea catechins and procyanidins. *Pharmaceut Biol*. 2004;42:84–93. https://doi.org/10.3109/13880200490893500.
90. Dai J, Wang G, Li W, et al. High-throughput screening for anti-influenza A virus drugs and study of the mechanism of procyanidin on influenza A virus-induced autophagy. *J Biomol Screen*. 2012;17(5):605–617. https://doi.org/10.1177/1087057111435236.
91. Zhuang M, Jiang H, Suzuki Y, et al. Procyanidins and butanol extract of Cinnamomi Cortex inhibit SARS-CoV infection. *Antivir Res*. 2009;82(1):73–81. https://doi.org/10.1016/j.antiviral.2009.02.001.
92. Maroli N, Bhasuran B, Natarajan J, Kolandaivel P. The potential role of procyanidin as a therapeutic agent against SARS-CoV-2: a text mining, molecular docking and molecular dynamics simulation approach. *J Biomol Struct Dyn*. 2020:1–16. https://doi.org/10.1080/07391102.2020.1823887.
93. Actis-Goretta L, Ottaviani JI, Keen CL, Fraga CG. Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. *FEBS Lett*. 2003;555(3):597–600. https://doi.org/10.1016/s0014-5793(03)01355-3.
94. Serra A, Macià A, Romero MP, et al. Bioavailability of procyanidin dimers and trimers and matrix food effects in in vitro and in vivo models. *Br J Nutr*. 2010;103(7):944–952. https://doi.org/10.1017/S0007114509992741.
95. Gansukh E, Kazibwe Z, Pandurangan M, Judy G, Kim DH. Probing the impact of quercetin-7-O-glucoside on influenza virus replication influence. *Phytomedicine*. 2016;23(9):958–967. https://doi.org/10.1016/j.phymed.2016.06.001.
96. Zhang DH, Wu KL, Zhang X, Deng SQ, Peng B. In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. *J Integr Med*. 2020;18(2):152–158. https://doi.org/10.1016/j.joim.2020.02.005.
97. Abian O, Ortega-Alarcon D, Jimenez-Alesanco A, et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. *Int J Biol Macromol*. 2020;164:1693–1703. https://doi.org/10.1016/j.ijbiomac.2020.07.235.
98. Pandey P, Rane JS, Chatterjee A, et al. Targeting SARS-CoV-2 spike protein of COVID-19 with naturally occurring phytochemicals: an in silico study for drug development. *J Biomol Struct Dyn*. 2020:1–11. https://doi.org/10.1080/07391102.2020.1796811.
99. Sharma A, Goyal S, Yadav AK, Kumar P, Gupta L. In-silico screening of plant-derived antivirals against main protease, 3CLpro and endoribonuclease, NSP15 proteins of SARS-CoV-2. *J Biomol Struct Dyn*. 2020:1–15. https://doi.org/10.1080/07391102.2020.1808077.
100. Saakre M, Mathew D, Ravisankar V. Perspectives on plant flavonoid quercetin-based drugs for novel SARS-CoV-2. *Beni Suef Univ J Basic Appl Sci*. 2021;10(1):21. https://doi.org/10.1186/s43088-021-00107-w.
101. Li C, Huang L, Sun W, et al. Saikosaponin D suppresses enterovirus A71 infection by inhibiting autophagy. *Signal Transduct Target Ther*. 2019;4:4. https://doi.org/10.1038/s41392-019-0037-x.
102. Cheng PW, Ng LT, Chiang LC, Lin CC. Antiviral effects of saikosaponins on human coronavirus 229E in vitro. *Clin Exp Pharmacol Physiol*. 2006;33(7):612–616. https://doi.org/10.1111/j.1440-1681.2006.04415.x.
103. Sinha SK, Shakya A, Prasad SK, et al. An in-silico evaluation of different Saikosaponins for their potency against SARS-CoV-2 using NSP15 and fusion spike glycoprotein as targets. *J Biomol Struct Dyn*. 2021;39(9):3244–3255. https://doi.org/10.1080/07391102.2020.1762741.
104. Dai JP, Zhao XF, Zeng J, et al. Drug screening for autophagy inhibitors based on the dissociation of Beclin1-Bcl2 complex using BiFC technique and mechanism of eugenol on anti-influenza A virus activity. *PLoS One*. 2013;8(4), e61026. https://doi.org/10.1371/journal.pone.0061026.
105. Saxena S, Kumar S, Hajare SN, Gupta S, Gautam S, Ghosh SK. 'BhAVI-23'-A spice-herb based dietary infusion possessing in-vitro anti-viral potential. *J Ayurveda Integr Med*. 2021;12(2):312–319. https://doi.org/10.1016/j.jaim.2020.11.005.
106. Silva JKRD, Figueiredo PLB, Byler KG, Setzer WN. Essential oils as antiviral agents. Potential of essential oils to treat SARS-CoV-2 infection: an in-silico investigation. *Int J Mol Sci*. 2020;21(10):3426. https://doi.org/10.3390/ijms21103426.
107. Tu Z, Gong W, Zhang Y, Feng Y, Liu Y, Tu C. Inhibition of rabies virus by 1,2,3,4,6-penta-O-galloyl-β-d-Glucose involves mTOR-dependent autophagy. *Viruses*. 2018;10(4):201. https://doi.org/10.3390/v10040201.
108. Liu G, Zhong M, Guo C, et al. Autophagy is involved in regulating influenza A virus RNA and protein synthesis associated with both modulation of Hsp90 induction and mTOR/p70S6K signaling pathway. *Int J Biochem Cell Biol*. 2016;72:100–108. https://doi.org/10.1016/j.biocel.2016.01.012. Epub 2016 Jan 18. PMID: 26794463.
109. Chiou WC, Chen JC, Chen YT, et al. The inhibitory effects of PGG and EGCG against the SARS-CoV-2 3C-like protease. *Biochem Biophys Res Commun*. 2021. https://doi.org/10.1016/j.bbrc.2020.12.106. S0006-291X(20)32299-3.
110. Chen RH, Yang LJ, Hamdoun S, et al. 1,2,3,4,6-Pentagalloyl glucose, a RBD-ACE2 binding inhibitor to prevent SARS-CoV-2 infection. *Front Pharmacol*. 2021;12:634176. https://doi.org/10.3389/fphar.2021.634176.
111. Choi JG, Lee H, Kim YS, et al. Aloe vera and its components inhibit influenza A virus-induced autophagy and replication. *Am J Chin Med*. 2019;47(6):1307–1324. https://doi.org/10.1142/S0192415X19500678.
112. Mpiana PT, Ngbolua KT, Tshibangu DST, et al. Identification of potential inhibitors of SARS-CoV-2 main protease from Aloe vera compounds: a molecular docking study. *Chem Phys Lett*. 2020;754:137751. https://doi.org/10.1016/j.cplett.2020.137751.
113. Dai JP, Li WZ, Zhao XF, et al. A drug screening method based on the autophagy pathway and studies of the mechanism of evodiamine against influenza A virus. *PLoS One*. 2012;7(8), e42706. https://doi.org/10.1371/journal.pone.0042706.
114. Varughese RS, Lam WS, Marican AABH, et al. Biopharmacological considerations for accelerating drug development of deguelin, a rotenoid with potent chemotherapeutic and chemopreventive potential. *Cancer*. 2019;125(11):1789–1798. https://doi.org/10.1002/cncr.32069.
115. Liao W, Liu X, Yang Q, et al. Deguelin inhibits HCV replication through suppressing cellular autophagy via down regulation of Beclin1 expression in human hepatoma cells. *Antivir Res*. 2020;174:104704. https://doi.org/10.1016/j.antiviral.2020.104704.
116. Min JS, Kim DE, Jin YH, Kwon S. Kurarinone inhibits HCoV-OC43 infection by impairing the virus-induced autophagic flux in MRC-5 human lung cells. *J Clin Med*. 2020;9(7):2230. https://doi.org/10.3390/jcm9072230.
117. Akkiz H. Implications of the novel mutations in the SARS-CoV-2 genome for transmission, disease severity, and the vaccine development. *Front Med*. 2021;8:636532. https://doi.org/10.3389/fmed.2021.636532.
118. Benvenuto D, Angeletti S, Giovanetti M, et al. Evolutionary analysis of SARS-CoV-2: how mutation of Non-Structural Protein 6 (NSP6) could affect viral autophagy. *J Infect*. 2020;81(1):e24–e27. https://doi.org/10.1016/j.jinf.2020.03.058. Epub 2020 Apr 10.
119. Grubaugh ND, Hanage WP, Rasmussen AL. Making sense of mutation: what D614G means for the COVID-19 pandemic remains unclear. *Cell*. 2020;182(4):794–795. https://doi.org/10.1016/j.cell.2020.06.040. Epub 2020 Jul 3.
120. Jackson WT. Viruses and the autophagy pathway. *Virology*. 2015;479–480:450–456. https://doi.org/10.1016/j.virol.2015.03.042. Epub 2015 Apr 6.
121. Maity S, Saha A. Therapeutic potential of exploiting autophagy cascade against coronavirus infection. *Front Microbiol*. 2021;12:675419. https://doi.org/10.3389/fmicb.2021.675419.
122. Andréa D. Fuzimoto, 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 Integra Med*. 2021:2095–4964. https://doi.org/10.1016/j.joim.2021.07.003.
123. Nair MS, Huang Y, Fidock DA, et al. Artemisia annua L. extracts inhibit the in vitro replication of SARS-CoV-2 and two of its variants. *J Ethnopharmacol*. 2021;274:114016. https://doi.org/10.1016/j.jep.2021.114016. Epub 2021 Mar 12.