

REVIEW ARTICLE

Antiviral plant-derived natural products to combat RNA viruses: Targets throughout the viral life cycle

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Significance and Impact of the Study: The COVID-19 pandemic has emphasised the need for new antiviral agents, including broad-spectrum drugs that can be rapidly deployed against emerging RNA viruses. Plant-derived natural products act on a diverse range of targets at different stages of the RNA virus life cycle suggesting they may be useful for the development of novel antiviral drugs. However, this review demonstrates that the modes of action of antiviral phytochemicals are often not well understood, and there are limited studies pertaining to *in vivo* efficacy, pharmacokinetic profile and toxicity, indicating that further research is required to evaluate the therapeutic potential of these compounds.

Keywords

antiviral, drug discovery, mechanism of action, natural product, phytochemical, plant, RNA virus.

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2021/0115: received 28 July 2021, revised 8 November 2021 and accepted 13 December 2021

doi:10.1111/lam.13637

Abstract

There is a need for new effective antivirals, particularly in response to the development of antiviral drug resistance and emerging RNA viruses such as SARS-CoV-2. Plants are a significant source of structurally diverse bioactive compounds for drug discovery suggesting that plant-derived natural products could be developed as antiviral agents. This article reviews the antiviral activity of plant-derived natural products against RNA viruses, with a focus on compounds targeting specific stages of the viral life cycle. A range of plant extracts and compounds have been identified with antiviral activity, often against multiple virus families suggesting they may be useful as broad-spectrum antiviral agents. The antiviral mechanism of action of many of these phytochemicals is not fully understood and there are limited studies and clinical trials demonstrating their efficacy and toxicity *in vivo*. Further research is needed to evaluate the therapeutic potential of plant-derived natural products as antiviral agents.

Introduction

Viral infections present a significant global health and socioeconomic burden (Gasparini *et al.* 2012; Dye 2014; Klepser 2014). This is exemplified by the emergence of the Coronavirus Disease 2019 (COVID-19) pandemic, which has caused >180 million infections and 4 million deaths (World Health Organization 2021) and is the largest pandemic since the 1918 influenza outbreak (Casella *et al.* 2021). RNA viruses are the most common source of emerging human infectious diseases, with notable zoonotic viral infections including coronaviruses, Zika, Ebola, chikungunya and dengue virus (DENV) (Carrasco-Hernandez *et al.* 2017). RNA viruses typically adapt more rapidly to new hosts owing to their relatively high mutation rates, given the error-prone nature of the viral RNA

dependent RNA polymerase (RdRp) and frequent recombination events (Holmes 2009). Understanding the threat of RNA viruses is key to limit and manage future global pandemics (Carrasco-Hernandez *et al.* 2017). There are 214 RNA virus species that cause infection in humans (Woolhouse and Brierley 2018), ranging in severity from mild to fatal in addition to causing severe chronic infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). There are approximately 38 million people living with HIV globally (UNAIDS 2020), and 71 million people with HCV, which causes severe liver cirrhosis in 10% of cases (World Health Organization 2020).

The current COVID-19 pandemic has emphasized a need for antiviral therapies that can be rapidly deployed against emerging viruses, particularly in the absence of an

effective vaccine. Antiviral drugs have traditionally been developed as single spectrum agents and there is a lack of broad-spectrum antivirals for treating emerging viral diseases (Vigant *et al.* 2015; Pattnaik and Chakraborty 2020). There has been increasing interest in repurposing of approved antiviral drugs for the treatment of new viral diseases and the discovery of broad-spectrum antiviral agents (Vigant *et al.* 2015; Harrison 2020; Pattnaik and Chakraborty 2020). The corticosteroid dexamethasone was the first approved treatment for hospitalized COVID-19 patients (Department of Health and Social Care 2020a) and the broad-spectrum antiviral remdesivir was also approved in the European Union (European Medicines Agency 2021), United Kingdom (Department of Health and Social Care 2020b) and United States (Rubin *et al.* 2020), while a range of other drugs are undergoing clinical trials (de Clercq and Li 2016; Normand 2020). The majority of currently licensed antivirals are antiretroviral drugs for HIV, or those that target chronic and latent diseases such as HCV (de Clercq and Li 2016), yet due to the rapid evolutionary rates of RNA viruses, antiviral resistance is a significant problem. For example, drug-resistant HIV has increased globally, and pan-resistant isolates have been reported (Puertas *et al.* 2020). A potential strategy to limit the development of viral escape is to target host proteins used for viral replication rather than virus itself (Müller *et al.* 2012). Broad-spectrum antivirals targeting conserved proteins are also hypothesized to have a higher threshold to resistance development; viral escape mutations in the coronavirus murine hepatitis virus (MHV) in the presence of remdesivir, which targets RdRp, had a significant cost to infection in a mouse model, suggesting conserved proteins are a promising target for broad-spectrum antivirals (Agostini *et al.* 2018).

Plants have an extensive history of use as traditional medicines, both for communicable and non-communicable diseases. Medicinal plants have been investigated as a source of novel therapeutic agents since the advent of modern drug discovery techniques (Beutler 2019). Plants produce an array of structurally diverse and often complex secondary metabolites with a range of functions; such compounds evolved for a particular biological function within plants, and they frequently exhibit biologically relevant privileged molecular scaffolds, which lends to their use as a reservoir for drug discovery (Rodrigues *et al.* 2016; Grigalunas *et al.* 2020). There has been a shift away from natural product research and towards high throughput screening of synthetic compounds for drug discovery over recent decades (Wright 2019). This can be attributed to the time-consuming nature of identifying bioactive constituents, difficulties with synthesis and derivatization of natural product

compounds, incompatibility with high throughput screening assays and the need for dereplication to remove previously characterized compounds (Atanasov *et al.* 2015; Beutler 2019; Wright 2019). A further challenge is that many phytochemicals exhibit poor solubility and bioavailability, requiring drug delivery strategies for their successful application as pharmaceutical products. The use of drug delivery strategies to improve the delivery of antiviral phytochemicals has been recently reviewed by Ben-Shabat *et al.* (2020). Conversely, natural products are still a significant source of new drugs, as an estimated 34% of approved new chemical entities between 2000 and 2014 were derived from natural products of plants and other organisms (Newman and Cragg 2016). Plants could therefore be a reservoir of novel antiviral agents, and there is a large body of literature reporting the antiviral activity of plants and their constituents. Many natural products are also known to exhibit antitumor activity which could be used to prevent or treat cancers associated with viral infections such as HCV and HIV (Fatima *et al.* 2019; Akhtar *et al.* 2020).

Biologically active plant-derived natural products are traditionally discovered using bioactivity guided fractionation. Crude extracts are first screened for their pharmacological activity, and fractionation steps are iteratively performed until the bioactive compounds are isolated and identified (Atanasov *et al.* 2021). There are a large number of published studies on the antiviral activity of plant crude extracts and their constituents, often based on plants used in traditional medicine. For example, bioactivity-guided fractionation was recently employed to determine the antiviral activity of 26 Nigerian medicinal plants against echoviruses E7, E13 and E19, with 10 plant extracts demonstrating antiviral activity. *Macaranga barkeri* methanolic extract was the most potent antiviral, with a 50% effective dose (EC₅₀) of 0.0017–0.028 ng ml⁻¹, of which the dichloromethane fraction (containing flavonoids) displayed the greatest activity with EC₅₀ values of 0.0075–0.0175 ng ml⁻¹ (Ogbole *et al.* 2018). Such studies often do not identify the active constituents of the crude extract. Plant extracts can be difficult to standardize, with variation in composition based on the growth conditions and extraction method (Wei *et al.* 2019), highlighting the importance of identifying active constituents. Moreover, a number of studies do not investigate beyond the initial determination of antiviral activity of crude extracts or their components, leaving the mechanism of action and molecular target undetermined. Advances in *in silico* approaches have enabled computational screening of natural product libraries for compounds that potentially bind to specific viral targets, which can then be subject to *in vitro* assays (Romano and Tatonetti 2019). Recently, computational methods have been employed extensively for

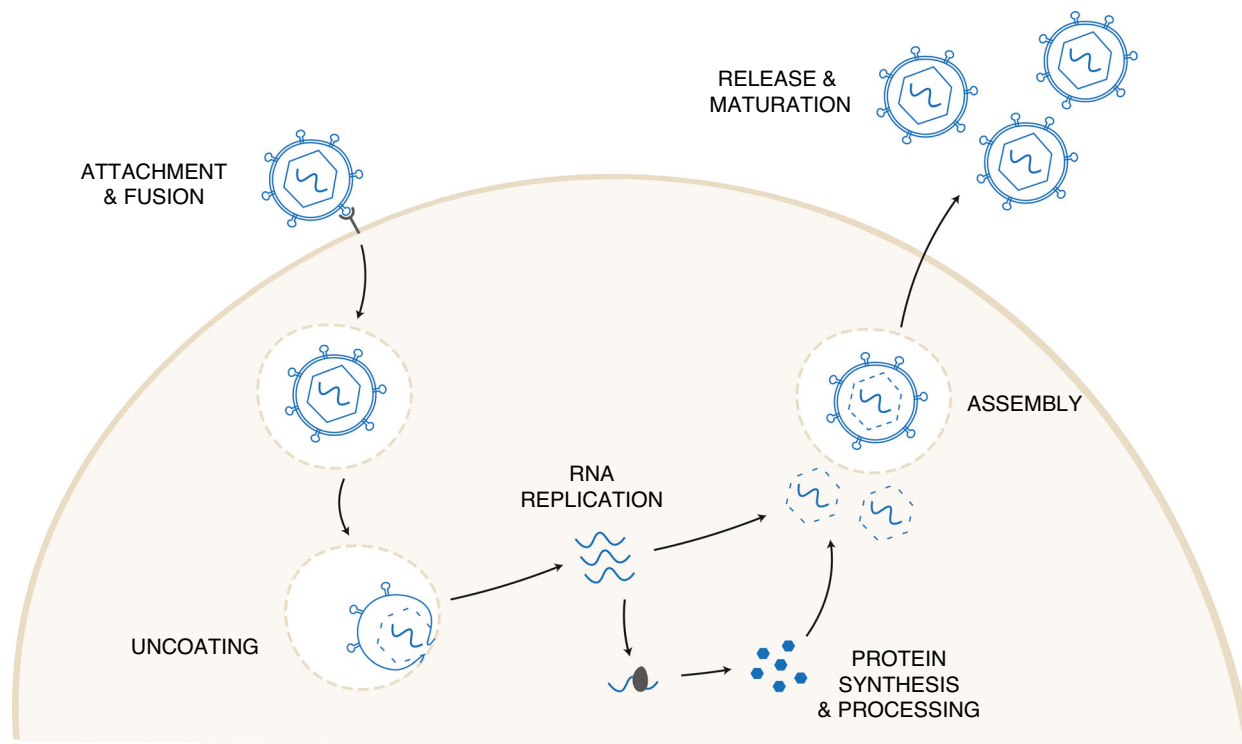


Figure 1 Stages of the viral life cycle.

natural products against potential severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets in response to the COVID-19 pandemic, however the antiviral activity of identified compounds *in vitro* are not reported in many of these studies (Kandeel *et al.* 2020; Tahir ul Qamar *et al.* 2020; Gyebi *et al.* 2021). There is a need for further in-depth studies to be performed against many of the antiviral plant products reported in the published literature to assess their importance in the development of antiviral agents. Despite this, the mode of action has been elucidated for a range of plant extracts with promising antiviral activity, targeting different steps throughout the viral life cycle and there are several examples of novel targets being identified, indicating that this is an under-researched area for further development. The aim of this article was therefore to discuss the antiviral activity of natural product compounds known to target specific stages of the life cycle of RNA viruses and their potential utility for the development of antiviral agents.


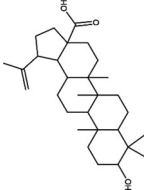
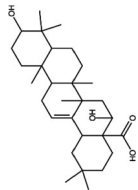
Viral life cycle

Viruses hijack the host cell and induce an array of changes to enable replication and production of progeny viruses. Briefly, the viral life cycle can be divided into three major stages: entry, replication, and assembly and

exit (Fig. 1). During entry, virions first attach to a specific host receptor and enter by endocytosis or fusion with the plasma membrane. The virus coat or capsid is then shed, either in the cytoplasm or in acidified endosomes, and the viral genome is released (Ryu 2017). The viral genome is then used for replication and transcription. The Baltimore system classifies viruses into seven genome types: double-stranded (ds) DNA, single-stranded (ss) DNA, dsRNA, positive sense (+) ssRNA, negative sense (–) ssRNA and +ssRNA viruses with a DNA intermediate (Flint *et al.* 2015; Aiewsakun and Simmonds 2018). The details of replication can vary significantly between RNA viruses depending on the genome type. In dsRNA viruses, the strand is transcribed into mRNA using virally encoded RdRp, and subsequently the mRNA is used to produce new copies of the genome. For +ssRNA viruses, the genome is directly translated into proteins, whereas in –ssRNA viruses require RdRp to transcribe the genome into mRNA. Finally, retroviruses use virally encoded reverse transcriptase (RT) to copy their +ssRNA genome into dsDNA, which is subsequently transcribed by host cell enzymes (Flint *et al.* 2015).

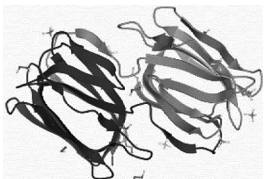
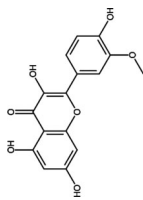
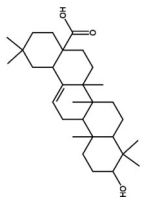
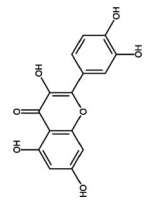
Replication can occur in different sites within the host cell, such as in the nucleus for influenza A virus (IAV) or HIV (Samji 2009), and in the cytoplasm in replication complexes for HCV and DENV (Ci and Shi 2021). Protein

Table 1 Antiviral activity (EC₅₀ or percentage inhibition) and cytotoxicity (CC₅₀ or percentage inhibition) of plant derived natural products targeting different stages within the viral life cycle (– Not defined; * Extract contains mixture of compounds)

Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
Attachment/ Fusion	BanLec	Lectin	PDB ID: 7KMU (Coves-Datson <i>et al.</i> 2021; Meagher and Stuckey 2021) 	HIV	Surface glycoprotein high mannose type glycans	0.48–2.06 nmol l ⁻¹	–	Swanson <i>et al.</i> (2010, 2015)
				HCV	Surface glycoprotein high mannose type glycans	9.5–20.8 nmol l ⁻¹	–	Swanson <i>et al.</i> (2015)
				IAV	Surface glycoprotein high mannose type glycans	0.06–11 µg ml ⁻¹	–	Swanson <i>et al.</i> (2015)
	H84T BanLec	Lectin		HIV	Surface glycoprotein high mannose type glycans	0.33–4.10 nmol l ⁻¹	–	Swanson <i>et al.</i> (2015)
	Betulinic acid	Triterpenoid		HCV	–	4.2%, 1 µmol l ⁻¹	~75%, 50 µmol l ⁻¹	Xiao <i>et al.</i> (2014)
	β-cyclodextrin-betulinic acid conjugates	Triterpenoid		HCV	–	37.1–88.2%, 1 µmol l ⁻¹	~15–85%, 50 µmol l ⁻¹	Xiao <i>et al.</i> (2014)
	Betulinic acid derivatives	Triterpenoid		HIV	gp120	0.31 to >40 µmol l ⁻¹	–	Sun <i>et al.</i> (2002)
	Echinocystic acid	Triterpenoid		HCV	HCV envelope glycoprotein 2	90%, 10 µmol l ⁻¹	>50 µmol l ⁻¹	Yu <i>et al.</i> (2013)
	β-cyclodextrin-echinocystic acid conjugates	Triterpenoid		HCV	HCV envelope glycoprotein 2	27.5%, 1 µmol l ⁻¹	0%, 50 µmol l ⁻¹	Xiao <i>et al.</i> (2014)
	Echinocystic acid-galactose conjugate	Triterpenoid		HCV	–	–40 to 86.5%, 1 µmol l ⁻¹	~1–90%, 50 µmol l ⁻¹	Xiao <i>et al.</i> (2014)
				IAV	HA	5 µmol l ⁻¹	>200 µmol l ⁻¹	Yu <i>et al.</i> (2014)

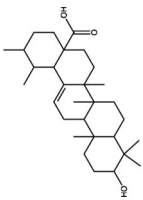
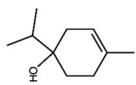
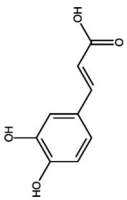
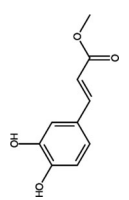
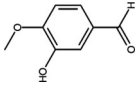
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Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
	<i>Fortunella margarita</i> EO Griffithsin	Lectin	* PDB ID: 2GTU (Ziolkowska and Wlodawer 2006; Ziolkowska et al. 2006) 	IAV HIV MERS-CoV SARS-CoV	– Surface glycoprotein high mannose type glycans Surface glycoprotein high mannose type glycans Surface glycoprotein high mannose type glycans	6.77 µg ml ⁻¹ 0.023–0.21 nmol l ⁻¹ 44.7–63.2%, 0.125 µg ml ⁻¹ 0.61–1.19 µg ml ⁻¹	239.54 µg ml ⁻¹ >500 nmol l ⁻¹ >2 µg ml ⁻¹ >100 µg ml ⁻¹	Ibrahim et al. (2015) Emau et al. (2007) Millet et al. (2016) O'Keefe et al. (2010)
	Isorhamnetin	Flavonoid		SARS-CoV-2	ACE2	47.7%, 50 µmol l ⁻¹	>200 µmol l ⁻¹	Zhan et al. (2021)
	Oleanolic acid	Triterpenoid		HCV HCV	HCV envelope glycoprotein 2 HCV envelope glycoprotein 2	60%, 10 µmol l ⁻¹ 22.4%, 1 µmol l ⁻¹	>50 µmol l ⁻¹ ~5%, 50 µmol l ⁻¹	Yu et al. (2013) Xiao et al. (2014)
	β-cyclodextrin-oleanolic acid conjugates	Triterpenoid		HCV	–	25.2–82.7%, 1 µmol l ⁻¹	~0–90%, 50 µmol l ⁻¹	Xiao et al. (2014)
	Quercetin	Flavonoid		IAV	HA2 subunit	1.93–7.76 µg ml ⁻¹	>250 µg ml ⁻¹	Wu et al. (2015)

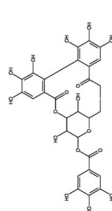
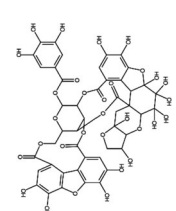
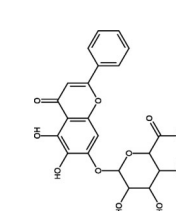
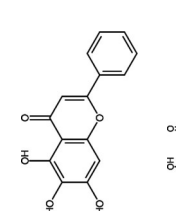
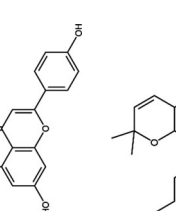
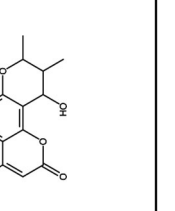
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Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
	Ursolic acid	Triterpenoid		HCV	–	13.8%, 1 μmol l ⁻¹	~90%, 50 μmol l ⁻¹	Xiao <i>et al.</i> (2014)
Uncoating	β-cyclodextrin-ursolic acid conjugates	Triterpenoid		HCV	–	13.6–62.6%, 1 μmol l ⁻¹	~1–90%, 50 μmol l ⁻¹	Xiao <i>et al.</i> (2014)
	Meliacine	Cyclic peptide	Cyclic peptide with aliphatic amino acids, MW 2.2–2.3 kDa *	Foot and mouth disease virus	Lysosome acidification	0.5 μg ml ⁻¹	>100 μg ml ⁻¹	Wachsman <i>et al.</i> (1998)
	Tea tree EO	–		IAV	Lysosome acidification	0.0006% (w/v)	0.025% (v/v)	Garozzo <i>et al.</i> (2011)
Polyprotein processing	Terpinen-4-ol	Monoterpenoid		IAV	Lysosome acidification	0.002% (v/v)	–	Garozzo <i>et al.</i> (2011)
	Caffeic acid	Hydroxycinnamic Acid		HIV	HIV Protease	90.2%, 1 mg ml ⁻¹	–	Wang <i>et al.</i> (2019)
	Ethyl caffeate	Hydroxycinnamic Acid		HIV	HIV Protease	100%, 1 mg ml ⁻¹	–	Wang <i>et al.</i> (2019)
	Isovanillin	Phenolic Aldehyde		HIV	HIV Protease	61.2%, 1 mg ml ⁻¹	–	Wang <i>et al.</i> (2019)

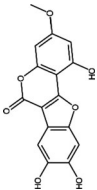
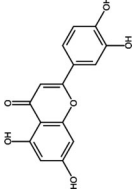
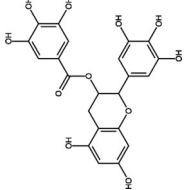
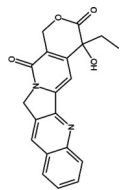
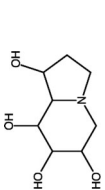

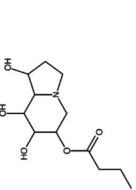
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Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
	Corilagin	Polyphenol		HCV	NS3 protease	13.59 µmol l ⁻¹	96.65 µmol l ⁻¹	Liu <i>et al.</i> (2012)
	Excoecarphenol D	Polyphenol		HCV	NS3 protease	12.61 µmol l ⁻¹	56.25 µmol l ⁻¹	Li <i>et al.</i> (2012)
	Shuanghuangjian Baicalin	Flavonoid		SARS-CoV-2 SARS-CoV-2	3CLPro 3CLPro	0.93–1.20 µl ml ⁻¹ 27.87 µmol l ⁻¹	>12.50 µl ml ⁻¹ >200 µmol l ⁻¹	Su <i>et al.</i> (2020) Su <i>et al.</i> (2020)
	Baicalin	Flavonoid		SARS-CoV-2	3CLPro	2.94 µmol l ⁻¹	>200 µmol l ⁻¹	Su <i>et al.</i> (2020)
	Apigenin	Flavonoid		HCV	RdRp NS5B	50 µmol l ⁻¹	75%, 100 µmol l ⁻¹	Manvar <i>et al.</i> (2012)
RNA Replication	Calanolide A	Coumarin		HIV	RT	0.08–0.50 µmol l ⁻¹	7.3–>10.0 µmol l ⁻¹	Buckheit <i>et al.</i> (1999)
	<i>Eclipta alba</i> aqueous extract	–	*	HCV	RdRp NS5B	95%, 130 µg ml ⁻¹	40%, 100 µg ml ⁻¹	Manvar <i>et al.</i> (2012)

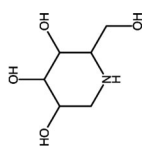
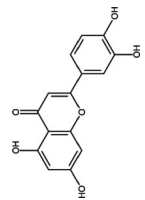
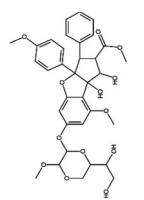
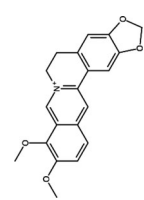
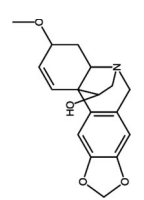
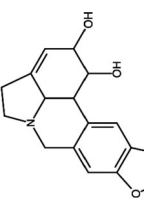
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Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
	Weddelactone	Courmestan		HCV	RdRp NS5B	80%, 50 µmol ml ⁻¹	79%, 100 µmol l ⁻¹	Manvar <i>et al.</i> (2012)
	Luteolin	Flavonoid		HCV	RdRp NS5B	50 µmol l ⁻¹	88%, 100 µmol l ⁻¹	Manvar <i>et al.</i> (2012)
	Green tea extract	–	*	SARS-CoV-2	NSp15 endo-ribonuclease	0.24 µg ml ⁻¹	–	Hong <i>et al.</i> (2021)
	Epigallocatechin gallate	Polyphenol		SARS-CoV-2	NSp15 endo-ribonuclease	0.092 µg ml ⁻¹	–	Hong <i>et al.</i> (2021)
Protein Synthesis	<i>Aegimelia indica</i> aqueous extract Camptothecin	– Alkaloid	* 	HCV Echovirus 71	NS5A phosphorylation Topoisomerase 1	90%, 8 mg ml ⁻¹ 90%, 10 µmol l ⁻¹	14 mg ml ⁻¹ >100 µmol l ⁻¹	Lin <i>et al.</i> (2018) Wu and Chu (2017)
	Castanospermine	Iminosugar		Zika virus	α-glucosidases	~60–90%, 1 µmol l ⁻¹	>100 µmol l ⁻¹	Bhushan <i>et al.</i> (2020)
	Curcumin	Polyphenol		EV71	Protein kinase Cδ	~50%, 10 µmol l ⁻¹	40–50%, 50 µmol l ⁻¹	Huang <i>et al.</i> (2018)
	Celgosivir	Iminosugar		DENV Zika virus	α-glucosidases α-glucosidases	5.17 µmol l ⁻¹ ~44–97%, 1 µmol l ⁻¹	>100 µmol l ⁻¹ >100 µmol l ⁻¹	Sayce <i>et al.</i> (2016) Bhushan <i>et al.</i> (2020)

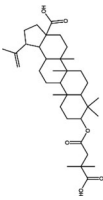
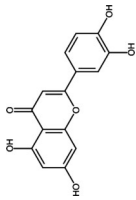
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Table 1 (continued)

Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
	Deoxy-nojirimycin	Iminosugar		Zika virus	α-glucosidases	~50–99%, 1 μmol l ⁻¹	>100 μmol l ⁻¹	Bhushan <i>et al.</i> (2020)
	Luteolin	Flavonoid		Coxsackie A16 EV71	–	7.4 μmol l ⁻¹ 10.31 μmol l ⁻¹	148.02 μmol l ⁻¹ 148.02 μmol l ⁻¹	Xu <i>et al.</i> (2014) Xu <i>et al.</i> (2014)
	Silvestrol	Flavagline		Ebola virus Human coronavirus 229E MERS-CoV Human rhinovirus A1	elf4a elf4a	0.8 nmol l ⁻¹ 3 nmol l ⁻¹	>10 nmol l ⁻¹ >10 nmol l ⁻¹	Müller <i>et al.</i> (2018) Müller <i>et al.</i> (2018)
Assembly	Berberine	Alkaloid		Poliovirus Zika virus IAV	elf4a elf4a MAPK/ERK pathway (ribo-nucleoprotein export)	1–3 nmol l ⁻¹ 100 nmol l ⁻¹ 20 nmol l ⁻¹ ~45%, 10 nmol l ⁻¹ 52 μmol l ⁻¹	>10 nmol l ⁻¹ >10 nmol l ⁻¹ >10 nmol l ⁻¹ ~70%, 50 nmol l ⁻¹ 1035 μmol l ⁻¹	Müller <i>et al.</i> (2018) Müller <i>et al.</i> (2018) Müller <i>et al.</i> (2018) Eigner <i>et al.</i> (2018) Botwina <i>et al.</i> (2020)
	Hemanthamine	Alkaloid		IAV	Ribo-nucleoprotein export	1.48 μmol l ⁻¹	50 μmol l ⁻¹	He <i>et al.</i> (2013)
	Lycorine	Alkaloid		IAV	Ribo-nucleoprotein export	<0.46 μmol l ⁻¹	20.9 μmol l ⁻¹	He <i>et al.</i> (2013)

(continued)

Table 1 (continued)

Life cycle stage	Extract/compound	Compound type	Structure	Virus	Target	EC ₅₀ /inhibition (%)	CC ₅₀ /inhibition (%)	Reference
Maturation	Bevirimat	Triterpenoid		HIV	Gag polyprotein	0.065 μmol l ⁻¹	>2 μmol l ⁻¹	Zhao <i>et al.</i> (2021)
	Luteolin	Flavonoid		DENV	Furin protease	4.36–8.38 μmol l ⁻¹	45.89 μmol l ⁻¹	Peng <i>et al.</i> (2017)

translation relies on host cell processes, with viruses possessing various strategies to shut down host protein translation to increase efficiency of viral protein translation (Walsh and Mohr 2011). Many viruses also encode highly conserved proteases that cleave the polyprotein and play a crucial role in replication (Sharma and Gupta 2017). Newly synthesized proteins are trafficked through the endoplasmic reticulum and Golgi apparatus where capsid assembly and genome packaging occurs to produce progeny viruses. Capsid assembly and genome packaging can be a sequential process, for example in picornaviruses, or occur simultaneously such as in adenoviruses (Ryu 2017). Progeny are subsequently released from the host cell, either by cell lysis (non-enveloped viruses) or budding (enveloped viruses), where the host cell lipid bilayer and transmembrane viral proteins surround the capsid as it is released from the plasma membrane (Willey *et al.* 2011). Finally, maturation occurs, where structural changes are induced to allow the virion to become infectious; for example, in HIV, the conformation of the viral capsid is altered by viral protease cleaving the Gag precursor protein into mature Gag proteins (Freed 2015).

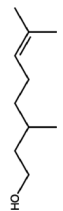
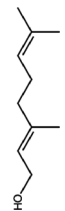
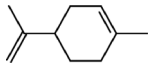
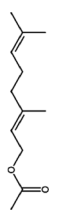
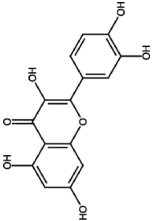
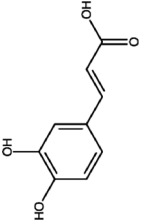
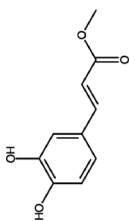
Plant-derived natural products targeting specific steps in the viral life cycle

Viral entry

Attachment and fusion

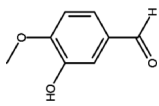
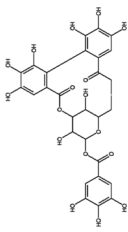
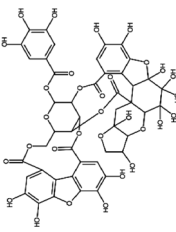
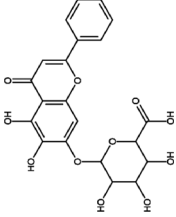
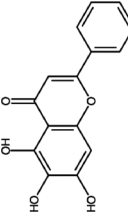
There are several examples of plant-derived natural products from a range of compound classes that inhibit the interactions between viral ligands and host cell receptors, suggesting they could potentially be developed as antiviral agents (Tables 1 and 2). Lectins are a structurally diverse group of non-enzymatic proteins that bind to carbohydrates (Mitchell *et al.* 2017). Binding to carbohydrates occurs via hydrogen bonds between polar residues and the hydroxyl groups of carbohydrates (Bellande *et al.* 2017). Lectins derived from plants have been extensively investigated as potential entry inhibitors (Table 1), primarily focusing on their applications as topical prophylactics against sexually transmitted diseases including HIV (Mitchell *et al.* 2017). BanLec, a lectin isolated from banana fruits, exhibited antiviral activity against a range of HIV-1 and HIV-2 isolates at an EC₅₀ ranging 0.48–2.06 nmol l⁻¹ (Swanson *et al.* 2010); the mechanism of action was attributed to BanLec binding to high mannose type glycans present on the viral envelope glycoprotein gp120, subsequently blocking glycan-mediated attachment to the host cell receptor, CD4, and/or co-receptors CCR5 and CXCR4 (Swanson *et al.* 2010; Hopper *et al.* 2017). Further development of BanLec was subsequently performed to ameliorate potential mitogenic effects of this

Table 2 Inhibition (IC_{50} or percentage inhibition) of target viral or host proteins by plant-derived natural products. (– Not defined; *Extract contains mixture of compounds)

Life cycle stage	Extract/compound	Compound type	Structure	Target	IC_{50} /inhibition (%)	Reference
Attachment/Fusion	Geranium EO	–	*	ACE-2	10.63%, 50 $\mu\text{g ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Lemon EO	–	*	ACE-2	24.79%, 25 $\mu\text{g ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Citronellol	Monoterpenoid		ACE-2	57.39%, 50 $\mu\text{mol ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Geraniol	Monoterpenoid		ACE-2	42.00%, 50 $\mu\text{mol ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Limonene	Monoterpene		ACE-2	28.22%, 50 $\mu\text{mol ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Neryl acetate	Monoterpenoid		ACE-2	7.61%, 50 $\mu\text{mol ml}^{-1}$	Senthil Kumar <i>et al.</i> (2020)
	Quercetin	Flavonoid		ACE2	4.48 $\mu\text{mol l}^{-1}$	Liu <i>et al.</i> (2020)
Polyprotein Processing	Caffeic acid	Hydroxycinnamic acid		HIV Protease	1.5 $\mu\text{mol l}^{-1}$	Wang <i>et al.</i> (2019)
	Ethyl Caffeate	Hydroxycinnamic acid		HIV Protease	1 $\mu\text{mol l}^{-1}$	Wang <i>et al.</i> (2019)

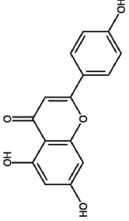
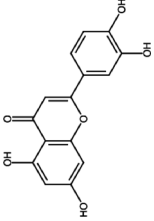
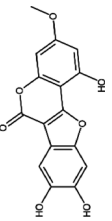
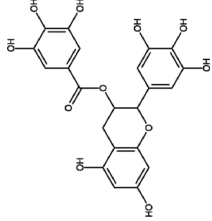
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Table 2 (continued)

Life cycle stage	Extract/compound	Compound type	Structure	Target	IC ₅₀ /inhibition (%)	Reference
	Isovanillin	Phenolic aldehyde		HIV Protease	3–5 $\mu\text{mol l}^{-1}$	Wang <i>et al.</i> (2019)
	Corilagin	Polyphenol		HCV NS3 Protease	3–45 $\mu\text{mol l}^{-1}$	Li <i>et al.</i> (2012)
	Excoecariphenol D	Polyphenol		HCV NS3 Protease	6–93 $\mu\text{mol l}^{-1}$	Li <i>et al.</i> (2012)
	Shuanghuangjian Baicalin	— Flavonoid		SARS-CoV-2 3CLPro SARS-CoV-2 3CLPro	0.01–0.09 $\mu\text{l ml}^{-1}$ 6.41 $\mu\text{mol l}^{-1}$	Su <i>et al.</i> (2020) Su <i>et al.</i> (2020)
	Baicalin	Flavonoid		SARS-CoV-2 3CLPro	0.94 $\mu\text{mol l}^{-1}$	Su <i>et al.</i> (2020)
RNA replication	<i>Eclipta alba</i> aqueous extract	—	*	HCV NS5B	11 $\mu\text{g ml}^{-1}$	Manvar <i>et al.</i> (2012)

(continued)

Table 2 (continued)

Life cycle stage	Extract/compound	Compound type	Structure	Target	IC ₅₀ /inhibition (%)	Reference
	Apigenin	Flavonoid		HCV NS5B	175.5 µg ml ⁻¹	Manvar <i>et al.</i> (2012)
	Luteolin	Flavonoid		HCV NS5B	11.3 µg ml ⁻¹	Manvar <i>et al.</i> (2012)
	Wedolactone	Coumestan		HCV NS5B	7.7 µg ml ⁻¹	Manvar <i>et al.</i> (2012)
	Green tea extract	–	*	SARS-CoV-2 Nsp15	2.54 µg ml ⁻¹	Hong <i>et al.</i> (2021)
	Epigallocatechin gallate	Polyphenol		SARS-CoV-2 Nsp15	0.74 µg ml ⁻¹	Hong <i>et al.</i> (2021)
	Peptide AIHIII	Peptide	7 amino acid peptide	HIV RT	274 nmol l ⁻¹	Seetaha <i>et al.</i> (2021)
	Peptide LIAVSTNIIIFW	Peptide	13 amino acid peptide	HIV RT	236.4 nmol l ⁻¹	Seetaha <i>et al.</i> (2021)

compound, which could lead to side effects in clinical applications; a BanLec mutant (H84T) substituting a histidine residue for threonine was significantly less mitogenic while retaining a similar EC_{50} against a range of HIV isolates ($0.33\text{--}4.10\text{ nmol l}^{-1}$) as wild type BanLec

($0.87\text{--}14.00\text{ nmol l}^{-1}$). Prophylactic vaginal application of H84T BanLec prevented HIV infection in a bone-marrow-liver-thymus humanized mouse model, with no detectable infections in the sample group, compared to infection in 50% of the control group (Table 3). BanLec

Table 3 *In vivo* efficacy of plant-derived natural products that target specific phases of the viral life cycle

Life cycle stage	Extract/compound	Virus	Model	Results	Reference
Attachment/Fusion	BanLec H84T (Lectin)	HIV	Bone-marrow-liver-thymus humanized mouse model; 75 μg , prophylactic vaginal administration	Prophylactic treatment with H84T significantly ($P \leq 0.05$) reduced the incidence of infection, with no HIV infection detected in the H84T treatment group compared to 50% of the control group	Swanson <i>et al.</i> (2015)
	Griffithsin (Lectin)	SARS-CoV	Mouse, 10 $\text{mg kg}^{-1}\text{ day}^{-1}$, intranasal administration	All mice in the griffithsin treatment group survived SARS-CoV infection compared to 30% survival in the no treatment group. Lung viral titres and weight loss were also significantly ($P \leq 0.05$) reduced by griffithsin treatment	O'Keefe <i>et al.</i> (2010)
RNA replication	Calanolide A (Coumarin)	HIV	Human, phase 1 clinical trial of healthy volunteers ($n = 47$); escalating doses for 5 days	Transient mild to moderate adverse effects were observed. Adverse effects and laboratory abnormalities were non-dose dependent. Absorption profiles and plasma levels of calanolide A varied significantly	Eizhamer <i>et al.</i> (2002)
Protein synthesis	Celgosivir (Iminosugar)	DENV	Mouse; 33 mg kg^{-1} three times daily, oral gavage	Significant reduction ($P \leq 0.05$) in viral RNA load. Marginal but non-significant ($P > 0.05$) reduction in infectious viral load	Sayce <i>et al.</i> (2016)
		DENV	Human phase 1b trial of dengue fever patients ($n = 50$); initial dose 400 mg, followed by 200 mg every 12 h for 4.5 days	Celgosivir was generally well tolerated. Treatment marginally but non-significantly ($P > 0.05$) reduced viral load compared to the control (-1.86 vs -1.64 virological \log_{10} reduction). No significant differences ($P > 0.05$) in fever burden or haematological and biochemical markers were observed	Low <i>et al.</i> (2014)
	UV-4B (Iminosugar)	IAV	Mouse; single dose 100–1000 mg kg^{-1} , oral gavage	Significant dose-dependent reduction in mortality at doses; $>250\text{ mg kg}^{-1}$ produced a 30–70% survival compared to 10% in the control group	Warfield <i>et al.</i> (2020)
		DENV	Mouse; single dose 250–1000 mg kg^{-1} , oral gavage	Significant dose-dependent reduction in mortality at doses, with 25–70% survival compared to 10% in the control group	Warfield <i>et al.</i> (2020)
Maturation	Luteolin (Flavonoid)	DENV	Mouse; 100 mg kg^{-1} , oral, four times per day	A significant ($P \leq 0.05$; twofold) reduction in viraemia compared to the control was observed 3 days post-infection	Peng <i>et al.</i> (2017)
	Bevirimat (Triterpenoid)	HIV	Human phase I and II clinical trial of HIV patients ($n = 24$); single dose of 75, 150 or 250 mg, oral administration	Significant reductions in HIV viral RNA load were observed in 150 and 250 mg groups ($0.46\text{--}0.47\text{ log}_{10}$ reductions) compared to the placebo (0.15 log_{10} reduction). No significant adverse effects were reported	Smith <i>et al.</i> (2007)

also demonstrated antiviral activity against HCV and H1N1, H3N2 and H5N1 strains of IAV, which also possess high mannose type glycans on their surface, suggesting that this compound has promise as a broad-spectrum antiviral (Swanson *et al.* 2015). Griffithsin is another well-characterized mannose-specific lectin, derived from red marine algae (*Griffithsia* sp.), with significant antiviral activity against HIV (Emau *et al.* 2007). Griffithsin also inhibits coronaviruses; the infectivity of Middle East respiratory syndrome coronavirus (MERS-CoV) was reduced by over 90% in MRC-5, Vero and Huh-7 cells by $2 \mu\text{g ml}^{-1}$ griffithsin (Millet *et al.* 2016), while severe acute respiratory syndrome coronavirus (SARS-CoV) was inhibited by griffithsin at an EC_{50} of 0.61–1.19 $\mu\text{g ml}^{-1}$ (O’Keefe *et al.* 2010). Griffithsin binds to the spike glycoprotein of coronaviruses, and while binding to the host cell receptor human angiotensin-converting enzyme 2 (ACE2) still occurs, viral entry is inhibited (O’Keefe *et al.* 2010). O’Keefe *et al.* (2010) also reported that griffithsin ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) significantly increased the survival of SARS-CoV infected mice to 100% compared to 30% in the control group (Table 3). These studies suggest that plant lectins could be useful for the development of broad-spectrum antiviral agents.

Pentacyclic triterpenoids are a large class of plant secondary metabolite reported to possess a range of biological activities (Bachořík and Urban 2021). Pentacyclic triterpenoids (Table 1) are hydrophobic isoprenoid compounds derived from oxidized squalene precursors (Cárdenas *et al.* 2019; Li *et al.* 2020). Echinocystic acid and oleanolic acid are members of the oleanane group of pentacyclic triterpenoids, which are derived from β -amyryn (Thimmappa *et al.* 2014), and have been reported to block viral attachment (Yu *et al.* 2013; Li *et al.* 2020). Yu *et al.* (2013) demonstrated that echinocystic acid and oleanolic acid inhibited the entry of HCV into Huh-7 cells by targeting HCV envelope glycoprotein 2, with respective EC_{50} values of 1.4 and $\sim 10 \mu\text{mol l}^{-1}$ and 50% cytotoxic concentration (CC_{50}) values of $>50 \mu\text{mol l}^{-1}$ (Yu *et al.* 2013). An echinocystic acid-galactose conjugate also inhibited IAV entry into MDCK cells at an EC_{50} of $5 \mu\text{mol l}^{-1}$ by binding to haemagglutinin (HA), thus disrupting its interaction with sialic acid on host cells (Yu *et al.* 2014). The insolubility of triterpenoids in water is a significant barrier to their potential use as antiviral agents and chemical modifications have been attempted to improve the bioavailability of triterpenoids. A range of triazole-bridged β -cyclodextrin (CD)–pentacyclic triterpene (betulinic acid, oleanolic acid, ursolic acid and echinocystic acid) conjugates inhibited the entry of HCV into Huh-7 cells and demonstrated improved water solubility and reduced hemolytic activity (Xiao *et al.* 2014). Derivates of betulinic acid, a lupane-type pentacyclic

triterpene (Bachořík and Urban 2021) have been shown to act as fusion inhibitors against HIV by targeting gp120 (Sun *et al.* 2002; Aiken and Chen 2005).

Quercetin, a flavonoid compound extracted from a wide range of plants, has been known to possess antiviral activity for decades (Colunga Biancatelli *et al.* 2020). Quercetin belongs to the flavonol group of flavonoids (Tables 1 and 2), possessing a backbone of 3-hydroxyflavone (D’Andrea 2015). There have been published reports of concentration-dependent inhibition of poliovirus, parainfluenza virus and herpes simplex virus type 1 in 1985 (Kaul *et al.* 1985). Since then, there has been a continued interest in the antiviral activity of quercetin; Wu *et al.* (2015) demonstrated that quercetin inhibited the infection of HCDK cells with IAV at an EC_{50} ranging 1.93–7.76 $\mu\text{g ml}^{-1}$. Time of addition assays demonstrated that quercetin inhibited the infectivity of IAV when used as a virus pre-treatment or co-treatment, suggesting that quercetin inhibits viral entry; in accordance expression of viral HA was limited when quercetin was added to infected cells up to 2 h post-infection. Quercetin also binds to the HA2 subunit of IAV, and inhibits HA-mediated haemolysis of erythrocytes, suggesting that quercetin may inhibit membrane fusion (Wu *et al.* 2015). More recently quercetin and related flavonoids have been investigated as a potential antiviral agent against SARS-CoV-2 using *in silico* approaches or *in vitro* protein assays (Derosa *et al.* 2021). Quercetin inhibited recombinant ACE2 at a 50% inhibitory concentration (IC_{50}) of 4.48 $\mu\text{mol l}^{-1}$ (Table 2). ACE2 acts as a receptor for SARS-CoV-2 spike protein and inhibition of ACE2 activity was proposed as a potential strategy to block binding and entry of SARS-CoV-2 (Liu *et al.* 2020). There is limited published research on the inhibition of SARS-CoV-2 infection by quercetin and other flavonoids, which is required to evaluate their potential utility as lead antiviral compounds. Zhan *et al.* (2021) reported that the flavonol isorhamnetin ($50 \mu\text{mol l}^{-1}$) reduced entry of a SARS-CoV-2 spike protein pseudotyped virus into ACE2-overexpressing HEK293 cells by 47.7%, indicating that isorhamnetin could inhibit ACE2-mediated entry; in contrast, quercetin did not significantly ($P > 0.05$) affect viral entry. Isorhamnetin did not display significant cytotoxicity up to $200 \mu\text{mol l}^{-1}$, suggesting that further investigation of its antiviral activity may be warranted (Zhan *et al.* 2021).

Essential oils (EOs) are volatile crude extracts of plants, predominantly containing terpenes, phenylpropanoids and related compounds, with well-established *in vitro* antibacterial and antifungal properties (Tariq *et al.* 2019). Research on the antiviral activity of EOs and their components appears to mainly comprise of preliminary antiviral screening assays. EOs derived from the fruits of

Fortunella margarita inhibited H5N1 IAV at an EC₅₀ of 6.77 µg ml⁻¹, compared to a CC₅₀ of 239.54 µg ml⁻¹ (Ibrahim *et al.* 2015); the active compounds and mechanism of action of this effect were not established. Recently EOs have been reported to inhibit ACE2. EOs derived from geranium and lemon (25–50 µg ml⁻¹) inhibited ACE2 protein concentrations (10.63–24.79% compared to control) and downregulated expression of ACE2 in HT-29 cells. Further screening of the major components of these EOs demonstrated that citronellol (50 µmol l⁻¹), geraniol (50 µmol l⁻¹), limonene (50 µmol l⁻¹), and neryl acetate (50 µmol l⁻¹) significantly reduced ACE2 levels by 7.61–57.39% compared to the control and did not exhibit cytotoxic activity at 100 µmol l⁻¹ (Senthil Kumar *et al.* 2020). The effect of these compounds against SARS-CoV-2 infectivity was not established, which is required to understand the significance of their ACE2 inhibitory activity.

Uncoating

There are few reports of plant-derived natural products that target uncoating (Table 1). Tea tree (*Melaleuca alternifolia*) EO and its terpenoid constituent terpinen-4-ol inhibited IAV, with respective EC₅₀ values of 0.0006 and 0.002% (v/v), compared to a CC₅₀ of 0.025% (v/v) against MDCK cells. Time-of-addition assays demonstrated that tea tree oil and terpinen-4-ol were active against IAV 1 h after viral adsorption and inhibited the acidification of MDCK lysosomes (Garozzo *et al.* 2011). Acidification of lysosomes is a key stage in the uncoating of IAV by prompting the fusion of the viral envelope and the endosomal membrane, thereby releasing the nucleocapsid into the cytoplasm (Guinea and Carrasco 1995; Garozzo *et al.* 2011). Similarly, meliacine, a cyclic peptide isolated from *Melia azedarach*, blocked lysosomal acidification in BHK-21 cells, resulting in the inhibition of foot and mouth disease virus uncoating (Wachsman *et al.* 1998), although the intracellular target that blocked acidification was not established.

Genome replication

Polyprotein processing

Upon entry into the host cell, the incoming RNA is translated and polyproteins processed to synthesize viral proteins involved in RNA replication. There are some examples of plant natural products that may inhibit proteases in the published literature (Table 1). A well-characterized target for coronaviruses is the main protease 3-Chymotrypsin-like protease (3CLPro) (Zhang *et al.* 2020), which cleaves nascent polypeptides into non-structural proteins required for genome replication (Mody *et al.* 2021). 3CLPro inhibitors have been of

significant interest for the development of antivirals for SARS-CoV-2, and as 3CLPro is highly conserved across coronaviruses, could also be active against SARS-CoV and MERS-CoV (Rathnayake *et al.* 2020). Shuanghuanglian is a Chinese traditional medicine extracted from the herbs *Lonicera japonica*, *Scutellaria baicalensis* and *Forsythia suspense* and used as a remedy for respiratory tract infections. Both oral liquid and lyophilized powder preparations of Shuanghuanglian were recently demonstrated to inhibit SARS-CoV-2 infection in Vero cells (EC₅₀ = 0.93–1.20 µl ml⁻¹) with activity being attributed to inhibition of 3CLPro. Investigation of the constituents of Shuanghuanglian identified that the two major flavonoid components baicalin and baicalein also showed 3CLPro-inhibitory activity (IC₅₀ = 6.41 and 0.94 µmol l⁻¹ respectively; Table 2) and respective EC₅₀ values against SARS-CoV-2 replication of 27.87 and 2.94 µmol l⁻¹; the CC₅₀ were >200 µmol l⁻¹ (Su *et al.* 2020). Molecular docking analysis suggested that two alkaloid compounds (10-hydroxyusambarensine and cryptoquinoline) and two pentacyclic triterpenoids (6-oxoisoguesterin and 22-hydroxyhopan-3-one) derived from an African plant library bind to SARS-CoV-2 3CLPro, suggesting that they could possess antiviral activity against SARS-CoV-2 by inhibiting viral protein cleavage (Gyebi *et al.* 2021). Quercetin was also identified as a potential inhibitor of SARS-CoV-2 3CLPro by molecular docking and *in vitro* enzyme inhibition assays (Abian *et al.* 2020). A range of plant compounds inhibit HIV protease (Ribeiro Filho *et al.* 2010) including the hydroxycinnamic acids ethyl caffeate and caffeic acid and isovanillin, a phenolic aldehyde compound at IC₅₀ values of 1.0–3.5 µmol l⁻¹ (Table 2) (Wang *et al.* 2019). Further examples of plant-derived protease inhibitors include the polyphenols corilagin and excoecariphenol D, which inhibited HCV NS3 protease (IC₅₀ = 3.45 and 6.93 µmol l⁻¹, respectively; Table 2) and RNA production in Huh-7.5 cells with respective EC₅₀ values of 13.59 and 12.61 µmol l⁻¹ (Li *et al.* 2012).

RNA replication

Retroviruses undergo reverse transcription of their RNA genome, catalysed by RT, before integrating into the host genome. RT is a key target for current anti-HIV drugs and as such has been the subject of interest for natural product drug discovery (Table 1). The coumarin compound calanolide A, present in the Malaysian tree *Calophyllum langigerum* strongly inhibits RT (Currens *et al.* 1996; el Sayed 2000; Eiznhamer *et al.* 2002). Buckheit Jr *et al.* (1999) reported that calanolide A inhibited HIV-1 with EC₅₀ values ranging 0.08–0.50 µmol l⁻¹; however, no antiviral activity was detected against HIV-2 or simian immunodeficiency virus, with EC₅₀ > 10 µmol l⁻¹). Calanolide A has subsequently been the subject of clinical

trials to assess its safety and pharmacokinetic profile as an anti-HIV agent, showing a reasonable safety profile with mild to moderate side effects in 47 healthy HIV negative participants (el Sayed 2000; Eiznhamer *et al.* 2002). Other plant-derived natural products that inhibit RT include two hydrolysed peptides AIHIILI and LIAVSTNIIFIVV from *Quercus infectoria* fruit peel, with IC₅₀ values of 236–274 nmol ml⁻¹ against RT (Table 2) (Seetaha *et al.* 2021).

The RNA genome of HCV is replicated by the RdRp non-structural protein 5B (NS5B). NS5B is a key target for the development of anti-HCV drugs, with several such drugs either approved for clinical use or under investigation (de Clercq and Li 2016). *Eclipta alba* has been used in traditional Indian medicine for the treatment of liver diseases. An aqueous extract of *E. alba* inhibited the replication of HCV *in vitro*, with a 90% reduction in viral RNA after treatment with 130 µg ml⁻¹. NS5B was also directly inhibited with an IC₅₀ of 11 µg ml⁻¹ (Table 2). Bioactivity-guided fractionation identified wedelolactone, luteolin and apigenin as active compounds, with respective IC₅₀ values against NS5B of 7.7, 11.3 and 175.5 µmol ml⁻¹ (Table 2). HCV replication was reduced by 80% upon treatment with 50 µmol ml⁻¹ wedelolactone (coumestan) and by 50% with 50 µmol ml⁻¹ luteolin (flavone) and apigenin (flavone). Cytotoxicity was observed at 50 µmol ml⁻¹, suggesting that the activity and selectivity of these compounds are limited (Manvar *et al.* 2012).

The infectivity of enterovirus 71 (EV71) and coxsackievirus A16 was reduced by luteolin, a flavone compound. EC₅₀ values of luteolin were 10.31 µmol l⁻¹ against EV71 and 7.40 µmol l⁻¹ against coxsackievirus A16, compared to CC₅₀ values of 148.02 µmol l⁻¹. Although the molecular target was not elucidated, mechanism of action studies suggested that luteolin targeted post-attachment stages of infection, where viral RNA expression in infected cells was suppressed upon treatment with luteolin (Xu *et al.* 2014).

A recent *in silico* study investigated potential inhibitors of SARS-CoV-2 RdRp from a library of 1664 chemicals previously approved for use in humans by the United States Food and Drug Administration. Although the *in vitro* antiviral activities of the compounds were not established, there were eight natural products identified with high molecular docking scores, including three glycosides (aloin, digoxin and sennoside B), two triterpenes (asiaticoside and glycyrrhizin), two flavonoids (quercetin and taxifolin) and one flavanone (neohesperidin dihydrochalcone). These results provide potential candidates for repurposing non-toxic and well-characterized compounds as SARS-CoV-2 replication inhibitors (Kandeel *et al.* 2020).

A natural product library of 28 compounds was screened for inhibitors of SARS-CoV-2 non-structural protein 15

(NSp15) endoribonuclease, an enzyme involved in the processing of viral RNA during replication. Green tea extract and its major constituent epigallocatechin gallate inhibited NSp15 with respective IC₅₀ values of 2.54 and 0.74 µg ml⁻¹ (Table 2), and inhibited SARS-CoV-2 infectivity against Vero cells at EC₅₀ values of 0.24 and 0.092 µg ml⁻¹ respectively (Hong *et al.* 2021).

Protein synthesis

There are several examples demonstrating the inhibition of various stages of protein synthesis (Table 1). *Aeginetia indica* is a parasitic plant found in Asia and historically used in folk medicine for liver diseases. An aqueous extract of *A. indica* was reported to possess antiviral activity against HCV; a dose of 8 mg ml⁻¹ reduced HCV infection of Huh-7.5.1 cells by 90% compared to a CC₅₀ of 14 mg ml⁻¹. It was concluded that *A. indica* targeted the translation and replication of HCV, as the expression of RNA and non-structural protein 3 (NS3) were reduced, and internal ribosome entry site-mediated translation activity was inhibited. Phosphorylation of NS5A protein at serine 235, a key post-translational event in the replication of HCV was significantly reduced, which was posited as the mechanism of action (Lin *et al.* 2018). The composition of the crude extract and the active compound were not elucidated, necessitating further study.

Another strategy for inhibiting viral protein synthesis is to target the host cell. Host cell proteins are less likely to mutate and lead to viral escape, compared to viral protein targets (Müller *et al.* 2012); conversely, there may be an increased risk of side effects from non-specific interactions when targeting host cells (Biedenkopf *et al.* 2017). Curcumin a polyphenolic compound extracted from turmeric (*Curcuma longa*) (Sharifi *et al.* 2020), was reported to inhibit HCV infection of Huh-7.5 cells (10–20 µmol l⁻¹) by upregulating the expression of heme oxygenase-1 and inhibiting PI3K-AKT signaling, thus inhibiting HCV protein expression (Chen *et al.* 2012). Curcumin (10 µmol l⁻¹) also inhibited EV71; pretreatment of HCT29 cells with 10 µmol l⁻¹ curcumin resulted in a significant ($P \leq 0.05$) decrease in viral titre and RNA expression at 6 to 9 h post-infection, whilst host cell viability was significantly ($P \leq 0.05$) increased. The expression of phosphorylated protein kinase Cδ was upregulated in EV71-infected HT29 cells, yet suppressed up to 6 h post-infection upon treatment with curcumin (Huang *et al.* 2018). Protein kinase Cδ is involved in a range of cellular processes including cell proliferation and has previously been shown to play a role in virus replication (Zhao *et al.* 2014). It was hypothesized that suppression protein kinase Cδ activation inhibits EV71 protein expression (Huang *et al.* 2018). Curcumin was significantly ($P \leq 0.05$) cytotoxic above 20 µmol l⁻¹ in both

studies (Chen *et al.* 2012; Huang *et al.* 2018), suggesting that its activity is not selective which may limit its utility. Indeed, curcumin interacts with a wide range of cellular targets including, for example, cytochrome P450 and NF- κ B (Huang *et al.* 2018).

Camptothecin, a quinoline alkaloid compound derived from the *Camptotheca acuminata* tree, has been extensively studied as an anticancer agent because of its topoisomerase I inhibitory activity (Li *et al.* 2017) but has more recently been investigated as a potential antiviral agent. Wu and Chu (2017) reported that camptothecin inhibited EV71; protein expression was suppressed in both replication-incompetent and replication-competent EV71, suggesting that both the transcription and translation of viral RNA were blocked by camptothecin. It was noted that the antiviral efficacy of camptothecin was relatively limited, with a 90% reduction in infectivity at a concentration of 10 $\mu\text{mol l}^{-1}$; investigations of its mechanism of action may enhance understanding of host-virus interactions and in turn could potentially lead to the development of novel antiviral agents for enteroviruses (Wu and Chu 2017).

Silvestrol is a flavagline compound isolated from *Aglaia* sp. trees that was first investigated as a potential anticancer agent but has more recently been demonstrated to possess antiviral activity (Oran Schulz *et al.* 2021). Silvestrol inhibits the ATP-dependent RNA helicase eukaryotic initiation factor 4a (eIF4a) by increasing its binding affinity to mRNA, thereby preventing protein translation (Biedenkopf *et al.* 2017; Oran Schulz *et al.* 2021). Silvestrol was demonstrated to inhibit Ebola virus at an EC_{50} of 0.8 nmol l^{-1} in Huh-7 cells and exhibited antiviral activity when added both before and after infection; no cytotoxicity was demonstrated up to 10 nmol l^{-1} . Promising antiviral activity was also reported against human coronavirus 229E, MERS-CoV, human rhinovirus A1 and poliovirus in MRC-5 cells, with respective EC_{50} values of 3, 1.3, 100 and 20 nmol l^{-1} and strong selectivity indices (SI) ranging >100 to 3330 (Müller *et al.* 2018). The infectivity of Zika virus was also significantly reduced (Elgner *et al.* 2018). These results suggest that silvestrol may be a candidate for the development of antiviral agents against a range of RNA viruses (Müller *et al.* 2018).

Iminosugars are a class of sugar analogues possessing a nitrogen atom in substitution of cyclic oxygen (Bhushan *et al.* 2020) that occur in plants and bacteria. Iminosugars competitively inhibit α -glucosidases, thereby inhibiting viruses that rely on the endoplasmic reticulum glycosylation pathway for correct folding of glycoproteins (Evans DeWald *et al.*, 2020). Iminosugars and their derivatives reportedly inhibit enveloped viruses, for instance celgosivir, a prodrug of the plant-derived iminosugar castanospermine, was demonstrated to inhibit DENV *in vitro*

($\text{EC}_{50} = 5.17 \mu\text{mol l}^{-1}$) and *in vivo*, where oral treatment significantly reduced viral load in a mouse model (Table 3) (Sayce *et al.* 2016). However, in phase 1b clinical trials celgosivir showed no significant reductions in viral load or fever in dengue fever patients compared to the placebo (Low *et al.* 2014). Celgosivir, castanospermine and deoxynojirimycin also significantly inhibited Zika virus infection *in vitro* at 1 $\mu\text{mol l}^{-1}$ (Bhushan *et al.* 2020). A single oral dose of *N*-(9'-methoxynonyl)-1-deoxynojirimycin hydrochloride salt UV-4B, a derivative of deoxynojirimycin, also significantly reduced mortality of influenza and DENV-infected mice (Table 3) (Warfield *et al.* 2020).

Assembly

Natural products targeting viral particle assembly have been less commonly reported in the published literature (Table 1). Two alkaloid compounds (lycorine and hemanthamine) derived from *Lycoris radiata*, a plant used in traditional Chinese medicine, block assembly of H5N1 IAV (He *et al.* 2013). Respective EC_{50} values of lycorine and hemanthamine were <0.46 and 1.48 $\mu\text{mol ml}^{-1}$, while the CC_{50} values against MDCK cells were 20.9 and 50 $\mu\text{mol ml}^{-1}$. The SI of these compounds was therefore moderate at 34 to >45, (where 2–10 was considered a weak SI, 10–50 moderate and >50 as a strong SI). Time-of-addition assays demonstrated that these compounds exhibited an antiviral effect when added post-infection rather than during pre-treatment or co-treatment. It was subsequently demonstrated that lycorine and hemanthamine blocked the export of viral ribonucleoprotein from the nucleus into the cytoplasm (He *et al.* 2013), where virion assembly occurs (Samji 2009). Recently, the alkaloid berberine was demonstrated to possess antiviral activity against IAV by impairing the export of ribonucleotides from the nucleus into the cytoplasm; this was attributed to the suppression of virus-induced phosphorylation of the mitogen-activated protein kinase/extracellular signal-related kinase 1 (MAPK/ERK1) signalling pathway (Botwina *et al.* 2020).

Maturation

Natural products may also target the maturation of virions (Table 1). The flavonoid luteolin was reported to inhibit DENV infection by blocking viral maturation. The infectivity of all DENV serotypes (DENV1–DENV4) was reduced against Huh-7 cells, where EC_{50} values were 4.36–8.38 $\mu\text{mol ml}^{-1}$ and the CC_{50} was 45.89 $\mu\text{mol ml}^{-1}$, giving an SI of 8.84 (Peng *et al.* 2017). Free virions isolated from the supernatant of infected cells contained elevated precursor membrane (prM) protein levels after

treatment with luteolin, suggesting that the virions were immature. DENV prM is a structural protein that, along with the Envelope (E) protein, encapsulate immature virions. Immature virions are trafficked to the Golgi apparatus where furin protease cleaves prM, producing a mature, infectious virion (Screaton *et al.* 2015; Peng *et al.* 2017). Further investigation showed that luteolin was a non-competitive inhibitor of furin protease (inhibitory constant of 58.6 $\mu\text{mol ml}^{-1}$). Luteolin exhibited a weak efficacy *in vivo* using a mouse model, where luteolin (100 mg kg^{-1} oral administration, four times per day) reduced viral titre in blood samples by twofold compared to the control (Table 3). This could potentially be attributed to the limited bioavailability of luteolin or low potency of the compound and suggests that compound optimization or drug delivery strategies are required (Peng *et al.* 2017).

In addition to fusion inhibition, betulinic acid derivatives block HIV maturation. Bevirimat (3-O-(3',3'-dimethylsuccinyl)betulinic acid) is a first-in-class HIV maturation inhibitor derived from betulinic acid (Zhao *et al.* 2021). This compound binds to Gag polyprotein, inhibiting the cleavage of the mature capsid protein p24 from capsid spacer protein-1 (p25), thereby preventing the development of mature infectious virions (Li *et al.* 2003; Martin *et al.* 2008). Phase I and II clinical trials were conducted in HIV patients ($n = 24$), suggesting that bevirimat (150–250 mg) significantly ($P \leq 0.05$) reduced HIV viral RNA load and did not produce significant adverse effects (Smith *et al.* 2007), however drug resistance was reported in up to 50% of patients due to the presence of polymorphisms in Gag at the capsid spacer 1 region in later studies (Adamson *et al.* 2010; Zhao *et al.* 2021). Investigation of alternative betulinic acid derivatives as potential HIV maturation inhibitors is ongoing (Zhao *et al.* 2021).

Conclusion and future perspectives

A wide range of plant-derived natural products have antiviral activity and act on a diverse range of targets at different stages of the virus life cycle suggesting they may be useful for the development of novel antiviral drugs (Table 1). Several plant extracts and compounds have demonstrated broad-spectrum antiviral activity *in vitro*. Future research needs to further investigate the antiviral efficacy of these natural products against a wider range of viruses and establish a fuller understanding of their mechanisms of action, including the molecular target of natural products, which will aid in the design of more potent derivatives (Aiken and Chen 2005). Computational methods have also revealed an abundance of compounds that interact with viral protein targets, and recently *in silico*

studies have been performed against SARS-CoV-2 (Kandeel *et al.* 2020; Tahir ul Qamar *et al.* 2020; Gyebi *et al.* 2021). The *in vitro* activity of compounds identified by *in silico* techniques are often not reported, however in order for natural products to be developed as antiviral drugs this efficacy data is paramount. Additionally, there are a number of *in vitro* studies that only report the antiviral efficacy of crude extracts without elucidating active constituents or mechanism of action which require more in-depth study. Natural products known to possess other bioactive properties have also seen increasing interest for their antiviral activity, including the repurposing of approved compounds which could be a strategy to accelerate the development of new antivirals (Kandeel *et al.* 2020). Another area of interest for future studies includes combinations of plant-derived natural products that exhibit synergistic interactions by blocking different stages of the viral life cycle, which could potentially enhance the potency of natural product compounds and limit the emergence of resistant isolates (Morán-Santibañez *et al.* 2018).

Plant natural products are often limited by poor solubility and bioavailability, and there are limited studies on the pharmacokinetic profile and *in vivo* toxicity of antiviral plant natural products. Derivatization has proven successful for increasing solubility (Xiao *et al.* 2014) and reducing toxicity (Swanson *et al.* 2015), while drug delivery systems have also been explored to improve the bioavailability of natural products (Wei *et al.* 2014; Ben-Shabat *et al.* 2020). Similar modifications may be an area of future study for recently identified antiviral plant compounds.

There are a limited number of *in vivo* studies (Table 3) on the antiviral activity of plant-derived natural products which are required to further understand the efficacy of compounds with promising *in vitro* activity (Tables 1 and 2). Few clinical trials related to antiviral natural products have been performed, and several recent systematic reviews have concluded that there is weak evidence that traditional herbal medicines are effective for viral diseases such as HIV, the common cold and SARS, due to the small number of studies and sample sizes, in addition to inappropriate methodologies for the majority of studies (Liu *et al.* 2005, 2012; Wu *et al.* 2007). As a result, there is a need for progression from *in vitro* efficacy tests and target elucidation towards *in vivo* studies and robust clinical trials for natural products to be developed as antiviral agents.

Author contributions

LO contributed to conceptualization, investigation and writing—original draft preparation. KL contributed to conceptualization and writing—review and editing. MS contributed to conceptualization, investigation and writing—review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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