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Natural Products and Antiviral Resistance

Erute Magdalene Adongbede[1], Janak Raj Khatiwada[2], Rishipal Rastrapal Bansode[2], Leonard Lamont Williams[2*]

[1]Department of Botany, University of Lagos, Lagos, Nigeria
[2]Center for Excellence in Post-Harvest Technologies, North Carolina A & T State University, Kannapolis, North Carolina, USA
Email: *llw@ncat.edu

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Abstract

Viral diseases are minacious with the potential for causing pandemics and treatment is complicated because of their inherent ability to mutate and become resistant to drugs. Antiviral drug resistance is a persistent problem that needs continuous attention by scientists, medical professionals, and government agencies. To solve the problem, an in-depth understanding of the intricate interplay between causes of antiviral drug resistance and potential new drugs specifically natural products is imperative in the interest and safety of public health. This review delves into natural product as reservoir for antiviral agents with the peculiar potentials for addressing the complexities associated with multi-drug resistant and emerging viral strains. An evaluation of the mechanisms underlying antiviral drug activity, antiviral drug resistance is addressed, with emphasis on production of broad-spectrum antiviral agents from natural sources. There is a need for continued natural product-based research, identification of new species and novel compounds.

Keywords

Drug Resistance, Resistance Mechanisms, Phytochemicals, Broad-Spectrum Drugs, Antiviral Agents

1. Introduction

Human history has recorded a significant number of high incidences of viral diseases ranging from the black death plaque that killed over 200 million between 1346 to 1353 [1] and the severe acute respiratory syndrome coronavirus disease 19 (COVID-19) with more than 6 million people worldwide [2]. The emergence of new and resistant viruses makes the failure rate of existing antiviral drugs high, causing the need for new and effective therapies. Antiviral drug resistance has economic consequences, and the World Bank says 3.8% of global gross domestic product could be lost to antimicrobial resistance by 2050 and

cause over 28 million people to go below poverty lines (WHO) 2022). Antiviral drug design targets either viral or cellular proteins, virus attachment, entry, viral DNA or RNA synthesis and or viral enzymes [3]. Multidrug-resistant strains of HIV have been successfully managed with combinational therapy, while the FDA has also approved some broad-spectrum drugs for treating viruses which are of natural parent origin [4]. Drugs such as acyclovir an analogue of guanosine a naturally occurring nucleoside potentially block viral replication and have been used with success [5]. These have however not been totally successful and emphasize the fact that new drugs are needed, and natural products hold the key to the problem. Some of the causes of antiviral drug resistance are-inherent propensity for viruses to mutate at very fast rates, misuse of antiviral agents and emergence of new viruses and serotypes [6]. In the past, natural products have served as valuable drug leads as their bioactive components present with pharmacophores that have been optimized or used directly as drugs. Naturally occurring compounds like Leonurine have shown broad-spectrum activity alongside capabilities like antiapoptotic and anti-inflammatory effects with potentials for use in treating viruses. Natural products are combinational as there a vast array of complex compounds with different activities still yet to be explored [7]. There are limited information on how specific natural products combat antiviral resistance, synergistic effects between these products and existing antiviral medications, the safety of natural products and development of resistance. It is important to note that nature holds to key to compounds that can effectively present as broad-spectrum agents against viruses. There is need to explore traditional and indigenous use of these natural products and fully understand how they can potentially be used as broad-spectrum antiviral drugs.

2. Mechanism of Action of Antiviral Drugs

Viral infections start with attachment and penetration transferring genetic material to the host cell nucleus using microtubules, making penetration and other infection processes good targets for antivirals (**Figure 1**) [8]. Some antiviral drugs block viral polymerase (phase 4 in **Figure 1**), e.g. zidovudine, nevirapine and maraviroc (inhibits HIV reverse transcriptase), lamivudine, entecavir, and telbivudine (nucleoside analogues), adefovir and tenofovir (nucleotide analogues) (Hepatitis B virus) [9]-[11]. Polytherapy (using more than one drug) is currently in use for HIV infections particularly with resistant strains [10]. Other antivirals that help others work very well providing synergistic effects e.g. oseltamivir, amantadine and ribavirin used for the treatment of H1N1 infection, adjudged to be more effective that the monotherapy [10].

Acyclovir: Acyclovir is activated viral thymidine kinase monophosphorylating it by viral thymidine kinase (TK) and then converted by cellular kinases to the active form -acyclovir triphosphate [5]. Acyclovir triphosphate inhibits HSV and VZV replication by competitive inhibition of viral DNA polymerase and by chain termination of viral DNA strands [5]. Ganciclovir on the other hand, uses the same mechanism of action as acyclovir it is not an obligate chain terminator,

instead causes a slowing down and subsequent cessation of viral DNA chain elongation [12].

Emtricitabine: Emtricitabine is a cytosine analog that functions as a nucleoside reverse transcriptase inhibitor (NRTI), its chemical structure (5-Fluoro-1-[2R,5S)-2-(hydroxymethyl)-[1,3] oxathiolane-5-yl] cytosine (FTC) is like that of lamivudine. It is used in combination of other antiretroviral drugs for the treatment of HIV-1, HIV-2 and hepatitis B virus (HBV) [13]. It can also be used in combination therapy as a pre-exposure prophylaxis to prevent HIV infection. The mechanism of action is like Acyclovir and has been used to efficiently restrain the synthesis of potential HIV DNA (**Figure 2**).

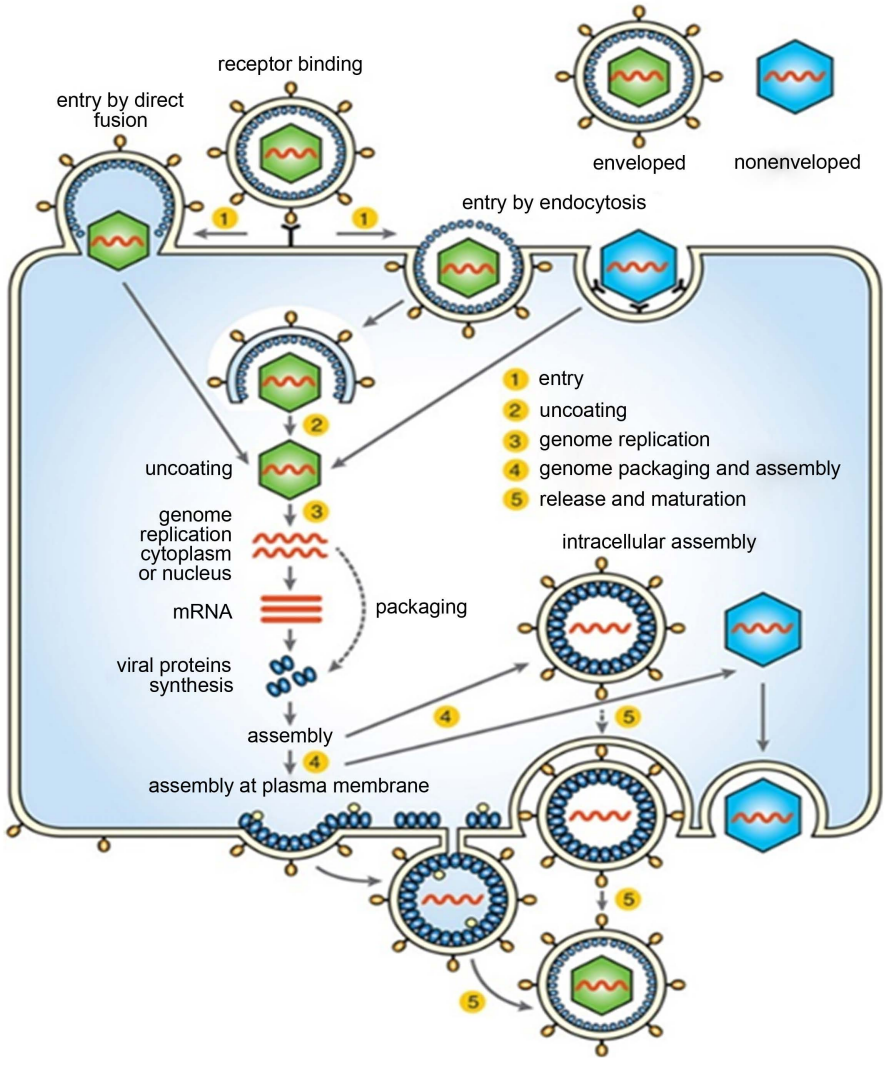
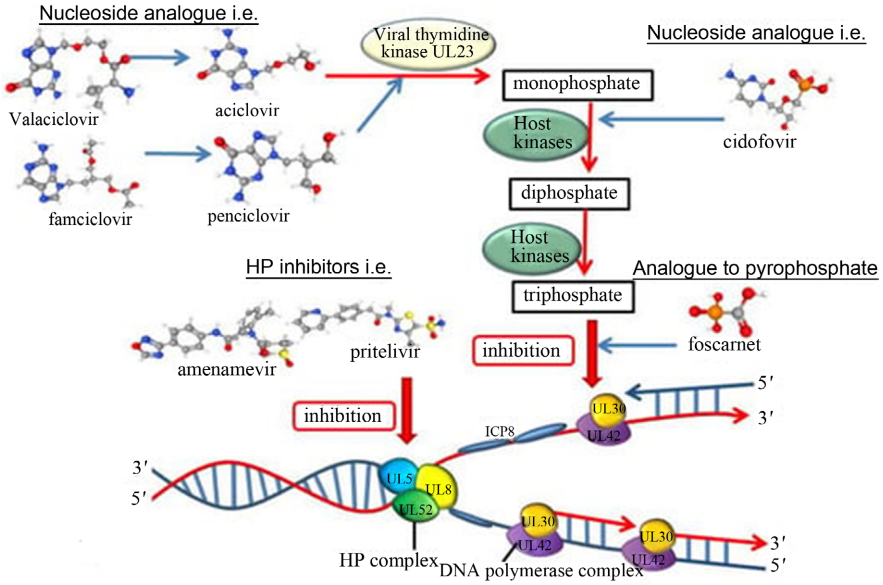


Figure 1. Simplified phases in the life cycle of a typical virus targeted by antiviral agents.

NRTIs work by blocking HIV's exploitation of reverse transcriptase, stopping the virus from transforming its RNA into DNA. In the absence of reverse transcriptase, HIV becomes incapable of replicating in the human body. The inhibition of reverse transcriptase promotes a reduction in the HIV viral load and an

increase in CD4+ T-cell count [13] [15]. One of the contraindications of this drug is that its monotherapy can lead to HIV drug resistance to antiretroviral therapy. Long-term use or prolonged exposure to this drug can lead to development of drug resistance [16]. Emtricitabine resistance is attributed to the M2041/V mutation with or without accompanying L180M and V173L mutations implying that there is cross-resistance to the drugs lamivudine and telbivudine. The drug Cabotegravir is to be used in combination with Rilpivirine for the treatment of HIV-1 in adults with suppressed viral load and no history of resistance to Cabotegravir or Rilpivirine [16].



UL5, UL8, UL52HSV-proteins forming a HP (helicase-primase) complex, UL30, UL42-HSV proteins forming a DNA polymarse complex, ICP8-single-stranded DNA binding proteine

Figure 2. Mechanism of action of nucleoside analogues like acyclovir [14].

2.1. Forscanet

Foscarnet is a pyrophosphate analog that inhibits DNA polymerase by simply preventing the pyrophosphate-binding site of the viral DNA polymerase. The mechanism of action of this drug uniquely helps combat antiviral drug resistance because viral resistance to nucleoside analogs is linked to mutations aimed at reducing or ending phosphorylation of the drug in virus-infected cells [17]. This drug is used to treat ganciclovir-resistant cytomegalovirus (CMV) infections in patients with acquired immunodeficiency syndrome (AIDS) or in transplant recipients. [18]. Clinically, Foscarnet is used exclusively for treatment of CMV-resistant and acyclovir resistant herpes simplex virus (HSV) and varicella zoster virus (VZV). In some cases, such as CMV-encephalitis, foscarnet could be used in combination with ganciclovir. These drugs have adverse effects such as nephrotoxicity and encephalopathy with acyclovir while ganciclovir is associated with myelosuppression, particularly neutropenia, nephrotoxicity and electrolyte abnormalities [18].

2.2. Drug Resistance

Drug resistance of infectious disease-causing agents like viruses is a critical concern to global public health. The use of antibiotics by humans is fraught with a major challenge of over-exposure of bacterial strains inherently present in the digestive and respiratory tracts indiscriminately to antibiotics [19]. Exposure to more antibiotics than needed by the body makes an individual a carrier of resistant pathogens that could potentially spread with time becoming problematic during surgeries or immunosuppression. Microorganisms -bacteria, mycobacteria, parasites, fungi and viruses have for several millions of years developed resistant mechanisms by developing genes that code for resistance to various threats such as heavy metals and antimicrobial compounds such as beta-lactams-penicillin, carbapenems, fluoroquinolones and sulphonamides [20]. Examples of AMR is seen in the buildup of resistance to colistin, an antibiotic that was once very effective for the treatment of multi-drug resistant Gram-negative bacteria is now widely reported as resistant with Enterobacteriaceae species [21]. In the treatment of fungal infections, drug resistance to azole derivatives like ketoconazole, fluconazole, itraconazole and voriconazole by *Aspergillus* species, *Cryptococcus neoformans*, *Candida* species and *Trichosporon beigelli* [22].

Multi-drug resistant *E. coli, Staphylococcus aureus, K. pneumoniae, S. pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* cause millions of deaths annually because of antimicrobial drug resistance [23]. According to WHO 2022 over 50% resistance was reported for bacterial infections caused by *Klebsiella pneumoniae* and *Acinetobacter* spp., globally [24]. In 2019, the total number of deaths attributed to antimicrobial resistance globally is 4.95 million and if urgent measures are not taken now, the estimate could double [24]. Chloroquine is a semisynthetic derivative of quinine, the first drug initially used to treat malaria and it relies on membrane-bound transporter proteins and became ineffective with time and replaced with artesunate and artemisinin due build up chloroquine resistant parasites [25] [26].

The economic consequences of drug resistance can be severe, and one estimate suggests that from 2000 to 2050 global losses due to resistant infection could be over 100 trillion US dollars [24]. According to this report, the World Bank estimates that up to 3.8% of the global gross domestic product could be lost because of AMR by 2050 and health care costs estimated to be up to I trillion US dollars by the year 2030 annually [24].

2.3. Antiviral Drug Resistance

There is always the fact that new viruses like Ebola, coronaviruses (SARS-CoV-2) can emerge further complicating managing viral infections. A good understanding of the mechanisms, factors and consequences of antiviral drug resistance is needed for developing effective therapies and mitigating its impact. It is therefore imperative that effective and safe drugs are used because infectious viruses like HIV, Hepatitis B and C can be potentially lethal leading to death and

as such natural products are under consideration as they are adjudged to be safe and effective [27]. The major reason given for the development of antiviral drug resistance is continued use of antiviral agents that do not clear the viral load and successful viral replication in individuals with low immune systems despite treatment [28]. A typical example is seen in the treatment of viral infections that takes prolonged periods, in some cases months and years as seen in HIV and the viruses that survive the treatment regime evolve and proliferate after mutating to more virulent strains [29]. Viral mutations cause changes in their genetic code particularly genes that code for viral proteins targeted by antiviral agents resulting in alterations in their amino acid sequences [6]. The exposure of viruses to different antiviral agents creates selective pressure on the virus population particularly with individuals that cannot clear viral loads with administered drugs, presenting a drug-resistant population [30]. The concentration of antiviral compounds in the system of patient might not be enough to clear the viruses completely for various reasons leading to insufficient drug concentrations which gives the virus selective advantage that could lead to the buildup of resistance to the drugs [29]. Cross-resistance is a factor that contributes to antiviral drug resistance for example if a drug targets reverse transcriptase and a virus exposed to it develops resistance to it, the progeny of this virus will be resistant to another member of this class of drug leading to cross-resistance. This therefore limits the effectiveness of multiple drugs against a resistant viral serotype [31]. Viruses can also develop resistance by modifying the target sites of antiviral drug compounds. This modification could involve structural changes in viral enzymes and receptors that make the binding sites on them unavailable to the drugs. E.g., with the treatment of HIV, resistance to reverse transcriptase inhibitor can occur through changes to the viruses' reverse transcriptase active sites. The virus could alter the expressions of functions of the activating enzymes rendering them useless and the drugs mechanism of action ineffective [32]. Viral recombination events occur very fast, and the pool of genes is so much that it is difficult to keep up with the exchange of materials and buildup of new variants. It is an established fact that gene reassortment might lead to over two hundred and fifty genetically viral progenies, making it apparent that influenza viruses are a great threat to public health [33]. Some viruses like HIV show long-term persistent infections providing extended time for development and selection of drug-resistant variants. Here, the main factor that leads to development of resistance by the viruses to drugs is the fidelity of viral replication, which is extremely high in double-stranded DNA viruses [34]. Enhanced drug efflux can cause of drug resistance as some viruses can develop strategies/mechanisms for pumping out antiviral compounds out of the infected cells and as such reducing the drugs concentration intracellularly [35].

Resistance to HIV-2 virus along with other direct-acting antivirals for treating viral infections like those of HBV, HCV, influenza, HSV, varicella zoster virus (VZV) and CMV is an established fact [36]. Common viral infections like the in-

fluenza viruses (H1N1) have shown resistance to drugs like oseltamivir used for treatment with the concomitant effect of loss of lives and economic loss from the cost of failed medication [37]. The consequences of antiviral drug resistance range from exposure to toxicity inherent in the use of second-line antivirals, to increased severity of disease symptoms and even death because of progression of viral infection when no effective alternative treatments are available.

2.4. Mechanism of Action of Antiviral Drug Resistance

Antiviral drug resistance is very problematic particularly with immunocompromised and immunosuppressed individuals because there is continued viral replication and the prolonged drug exposure results in the development of resistant serotypes. Resistance to drugs like acyclovir has mostly been with immunosuppressed and immunocompromised individuals. The mechanism of HSV to acyclovir is through viral thymidine kinase (TK) or DNA polymerase mutation. TK mutation could result in deficient virus not responsive to the drug of choice and resistance has also been linked to TK substrate specificity. Cross-resistance to other drugs like penciclovir is expected with TK deficient mutants [12].

In the hospitals, acyclovir-resistant HSV infections are treated with foscarnet with success. CMV resistance to the drug ganciclovir is characterized by mutation in the viral gene UL97 kinase, which is capable of decreasing phosphorylation of ganciclovir, rendering it impotent. Drugs approved for treatment of hepatitis B infections are nucleoside analogs (lamivudine) and nucleotide analogs (tenofovir) and these target the HBV DNA polymerase and these drugs require prior phosphorylation by cellular enzymes. HBV infections are treated using combinational therapy because of antiviral drug resistance. Mutations in the reverse transcriptase domains of the viral polymerase gene are responsible for development of resistance to these drugs. High degrees of lamivudine resistance has been attributed to tyrosine-methionine-aspartate-aspartate motif in the C domain of the polymerase gene [12].

The resistance of HIV to drugs like zidovudine and lamivudine was prevalent and this led to the introduction of combination therapies for the treatment of HIV-1 and HIV-2 infections. The main mechanism of resistance is mutation in the reverse transcriptase gene which affects the drug binding sites and polymerase activity [9]. Fostemsavir (formerly GSK-3684934) is a first-in-class oral attachment inhibitor binding to gp 120. It is to be used in combination with other antiretroviral drugs for the treatment of HIV-1 infection in heavily treatment-experienced adults with multidrug-resistant HIV-1 infection in whom current treatment failed due to resistance, intolerance, or safety considerations. Records show that Fostemsavir-associated resistance does not result in cross-resistance to other entry or attachment inhibitors such as ibalizumab and maraviroc [16].

Ibalizumab is a humanized monoclonal antibody and noncompetitive CD4 post-attachment inhibitor with approval for the treatment of patients with mul-

ticlass drug-resistant viruses. This is because the mechanism of attachment for this drug requires a previous attachment of HIV-gp 120 to the CD4 receptor and it therefore does [38]. There are no recorded mutations to this drug and as such genotypic testing to predict resistance is not recommended [16].

The use of nano-compounds in designing antiviral agents will also potentially improve the efficiency of the drugs, time of action and ensure that dosages are sufficient to hit target sites minimizing the risk of resistance due to prolonged exposure [17]. There are seven main classes of antiviral drugs currently been used based on broad spectrum activities, and they are neutralizing antibodies, neutralizing recombinant soluble human receptors, antiviral CRISPR/Cas systems, interferons, antiviral peptides, antiviral nucleic acid polymers and antiviral small molecules [39]. The use of RNA-targeted therapies that interfere with viral RNA replication or its translation will potentially disrupt essential RNA functions that can hamper the progression of the disease in the host cells [39].

2.5. Natural Products and Antiviral Drug Discovery

The bioactive compound in plants, algae, animals, marine organisms, and microbes have very complex and intricate chemical compositions because they are derived from natural selection or evolutionary pressures of adaptation, making these organism huge deposits for medicinally valuable molecules. Paclitaxel isolated from the plant *Taxus brevifolia* in the early 70s is currently being used for treating cancer [40]. The compound Leonurine, extracted from the plant *Herb leonuri* displays broad-spectrum activities such as antioxidant, antiapoptotic and anti-inflammatory potentials [7]. The popularity of drug discovery from natural sources is clear from the fact that more than 60% of low molecular weight drugs discovered in the past two decades are from natural source [41].

Antiviral drug discovery is based on interception and stoppage of viral growth at various stages directly from outside the cell, disrupting attachment to cellular receptors. Cholesterol and some rare forms of lipids utilized for viral replication and apoptotic processes could be targets for combating viral infection during antiviral drug discovery [42]. For example, with human enteroviruses, several compounds (such as benzothiopene derivatives) have been designed to fit into special hydrophobic pocket, replacing the aliphatic fatty acids that normally house the viral particle [42]. This pocket is important because it is close to the receptor binding area and as such capable of targeting the ability to bind to receptors at such sites [43]. Virus groups that use similar receptors to bind, could be targeted with specific compounds preventing viral infection [44]. Some drugs target cytoplasmic endosomes used by viruses as their main portal for entering cells and are particularly effective against non-enveloped viruses [45]. Targeting translation and transcription mechanisms of RNA-based viruses could be an effective way of stopping their infection process [46]. Strategies for antiviral drug discovery should also involve targeting membranous structures used by DNA viruses for replication at the surface [47].

2.6. Natural Products with Antiviral Activities

The synthetic antiviral agents initially used were toxic at therapeutic doses and as such the natural products seem to be better options. Interest in the use of natural products as antiviral agents was initially limited by the fact that screening, purification, and identification of antiviral compounds in crude extracts was very cumbersome. The renewed surge in the use of natural compounds as a source of antiviral agent stems from the fact that the technology for detection, isolation and structural characterization of these compounds has greatly improved. There are bioactive compounds from natural sources and derivatives of these compounds that have antiviral properties [48]. The phytochemicals (secondary metabolites) that have been implicated in antiviral activity are listed in Table 1 below and broadly, these include alkaloids, flavonoids, glycans, organic acids, terpenoids, phenolics, terpenoids, tannins anthocyanins and flavones and potentially have broad spectrum effects with their activities varying with the species from which they are isolated or extracted [49].

Table 1. Antiviral activity of some natural products.

Natural Product Parent Source	Bioactive Compounds	Activity
Taraxacum officinale	Flavonoid	Inhibition of HCV NS5B replicase [50]
Plantago major L.	Chlorogenic acid, p-coumaric acid, chlorogenic acid	Inhibits viral adsorption processes [51] [52]
Vitis vinifera (Grape Seeds)	Phenolic Compounds	Suppression of HVC, Hepatitis A virus activity
Ocimum basilicum	Ursolic Acid	Inhibits Coxsackievirus
Camellia sinensis	Epigallocatechin-3-gallate (EGCG); Catechins	Broad-spectrum activity: disrupts viral structure, inhibits viral infection and replication [53]
Panax (quingquefolius) L. ginseng	Ginsenoides	Targets host cellular factors inhibiting murine gamma herpes virus 68 (MHV-68) [54]
Curcumin longa L.	Curcumin	Broad-spectrum Antiviral Activity
Nostoc ellipsosorum	Ascyanovirin N	Prevents viral entry to cells
Euphorbia kansui	Ingenoil, Bryostatin, Prostatin	Reverses latency of [55] viruses like HIV (LRAs)
Theobromao cacao	Procyanidin, C1-flavanoids	Acts as Latency-Reversing Agents (LRAs)
Ganoderma pfeifferi	Triterpenoids (ganoderone A and lucialdehyde B, ganodermadiol)	Inhibits by Pretreatment effects and protection of cells [56]
Leiurus quinguestiatus hebraeus (scorpion-deathstalker)	Charybdotoxin, Scylla toxin	Inhibits viral replication by binding to the HVI gp120 glycoprotein, preventing viral entry to host cells [57].
Trimeresurus stejnegeri (Chinese tree green viper)	L-amino acid oxidase (LAAOI)	Anti-HIV activity, inhibits infection and replication, formation of syncytium at nanomolar concentrations [57].
Apis mellifera (honeybee)	Melittin (amphipathic peptide)	Virucidal effect against HIV-1 virus in VK2 cell line [58]

3. Secondary Metabolites with Antiviral Properties

Alkaloids: Amines produced by plants and other organisms potentially display antiviral effects. Records show that alkaloids isolated from the seeds of the plant *Peganum harmala* inhibited the influenza A virus replication in Madi-Darby canine kidney (MDCK) cells preventing viral polymerase activity [59]. The alkaloids Caulerpin extracted from the marine alga *Caulerpa lamouroux* (Caulerpales) disrupted the α and β phases of the replication cycle of the Herpes simplex virus type-1 (HSV-1) [60].

Quinones: Zeylanone epoxide, a naphthoquinone compound extracted from *Diospyrios anisandra* can potentially block extra-nuclear transport of viral nucleoprotein, reducing viral titre of the influenza-A and -B viruses [61]. Another compound acetylshikonin directly inactivated viral particles at exceptionally low concentrations blocking the uptake and or entry of the coxsackie virus A16 (CVA16) in *in vitro* experiments [62]. Virucidal effects of quinone compounds have been attributed to the formation of a keto-phenol system and also increasing number of hydroxyl groups on the same benzene ring [63].

Flavonoids: these are water-soluble compounds in plants with potential antioxidant activities linked as antiviral agents. Quercetin has been used in combination with drugs like Famipiravir to treat patients critically ill with neo-coronary pneumonia successfully [64] [65]. Baicalin is a flavonoid glucoside from *Scutellaria baicalensis* with strong intracellular antiviral activity affecting post entry phase of the Chikungunya virus replication [66]. Hesperidin is bioflavonoid capable of interfering with different stages if invasion and replication of coronavirus [67].

Terpenoids: Naturally occurring plant hydrocarbons such Artesunate a sesquiterpene lactone have been used to control secretion of HbsAg (surface antigen) and reduce gene expression in Hepatitis B virus [68] [69]. The combination of terpenoid-myricetin and acetylated galactose strongly inhibited Ebola virus [70]. The presence of hydroxyl and carboxyl groups to triterpenoids like ursolic acid and oleanolic acid significantly increased antiviral activity against Hepatitis C virus [71]. Endophytic fungi like Phoma sp. have bioactive terpenoid compound 14-nordrimane sesquiterpenoid with strong antiviral activity [72]. The compound Tatanan A is a new sesquiterpene lignan that can suppress translation and early RNA synthesis of the DENV-2 virus [73].

Catechin: a flavanol in green tea extracts shown to be capable of reducing viral FCV-F9 infection and further analysis by HPLC that epigallocatechin gallate is the major compound that exhibited the best antiviral activity with IC_{50} of 12 mg/mL and low cell cytotoxicity [74].

Polyphenols: Pomegranate-derived polyphenols exhibited greater activity against MNV-1, and reduced plaque formation of MNV-1 by 1.30 - 3.61 $\log_{10}$ PFU/mL [75].

Saponins: These are triterpene glycosides abundant in plants like red ginseng and records indicate that, MNV-1 titers were significantly reduced in cells pre-

treated with red ginseng extract, but not in cells that were treated at, or following, the time of viral challenge. These outcomes suggest that red ginseng extract and ginsenosides have significant anti-norovirus properties [76].

Organic Acids: Organic acids like ferulic acid, cinnamic acid, chlorogenic acid and caffeic acid are natural compounds with antiviral activity [77] Caffeic acid active against HCV replication and mechanism of action is activation of the Kelch-like ECH-associated protein 1/Nuclear factor. The acid stops severe fever associated with thrombocytopenia syndrome virus (SFTSV) infection was through suppression of attachment of virus to cells [78]. Other research found out that caffeic acid displayed higher virucidal activity than chlorogenic acid from elderberry extracts against the human coronavirus NL63 [79].

Oregano Essential Oil: Essential oils of the plant Oregano inhibited FCV virus reducing the viral titer by 3.75 log $TCID_{50}$/mL [80]. Carvacrol the essential component of Oregano oil inactivated MNV-1 within 1 h of exposure and significantly reduced MNV-1 infectivity (0.95 and 1.28 log TCID50/mL, respectively) within 15 min of exposure [81].

4. Antiviral Activity of Primary Metabolites

4.1. Antiviral Polysaccharides from Algae

Polysaccharides of algal origin with antiviral effects abound and they include carrageenan, alginate, fucan, laminaran, and naviculan [82]. These polysaccharides have different mechanisms of action as antiviral agents ranging from inhibiting binding and internalization of viruses to host cells to suppressing viral DNA replication and protein synthesis [82].

4.1.1. Red Algae

Carrageenan: Previous studies have shown that this sulphated polysaccharide extracted from red algae from the genera *Chondrus, Gigartina, Hypnea* and *Eucheuma* inhibited viral activity (rhinovirus, human papillomavirus) by preventing adsorption and attachment to host cells [83]. Carrageenan isolated from *Gigartina skottsbergii* potentially active against influenza virus, DENV, HSV-1, HSV-2, HPV, HRV and HIV viruses. They are extremely effective against sexually transmitted HPV types that lead to cervical cancer and genital warts (human papillomavirus) [83]. Extracts from *Polysiphonia denudata* (a red marine algae) showed inhibitory effects by preventing adsorption and intracellular stages of the replication of HSV-1 and HSV-2 at an effective concentration of 8.7mg/ml [84]. Terpenoid compounds extracted from red macroalgae -*Dictyota pfaffi* and *Dictyota menstrualis* also demonstrated anti-HIV activity with low toxicity [84].

Galactan: Some Galatans (extracellular sulfated polysaccharides structurally different from carrageenan) extracted from the following red algae-*Callophyllis variegata* (an edible seaweed), *Agardhiella tenera*, *Schizymenia binderi*, *Cryptonemia crenulata* recorded activity against HSV-1, HSV-2, HIV-1, HIV-2, DENV

and HAV viruses [83]. Mechanism of action in HIV-1 & 2 infection is prevention adhesion of the virus to cell and attachment of gp 120 on CD4+ T cell receptor to HIV-1 gp 120 [85].

4.1.2. Brown Algae

Alginates: The marine polysaccharide 911 is an alginate polysaccharide that showed strong activity against HIV-1 at both chronic infection stages of H9 cells and acute infections of MT4 cells in both *in vitro* and *in vivo* experiments [86].

Fucan and Fucoida. Sulfated fucans (high molecular weight polysaccharide) from *Dictyota mertensii, Lobophora varietgata, Fucus vesiculosus and Spatoglissum shroederi* have been used to prevent HIV infection through blocking of the reverse transcriptase activity-[87]. Another fucan polysaccharide isolated from *Cladosiphon akamuranus* inhibited DENV-2 infection in BHK-21 cell line, while MC26 is a fucose polysaccharide isolated from *Sargassum piluiferum* with strong anti-influenza virus activity and low cytotoxicity [88].

Fucoidans are biologically active against HIV, HSV-2, Dengue virus and cytomegalovirus blocking interaction of viruses with cells preventing viral induced syncytium formation [88]. Fucoidans extracted from species like *Stoechospermum marginatum* and *Cystoseira indica* showed potential antiviral activity against Newcastle disease in Vero cell line [88]. Polysaccharides (ulvan and fucoidan) from sea weeds *Ulva clathrata* and *Cladosiphon okamuranus* could inhibit viral attachment and cell fusion in Newcastle Disease virus (NDV) infection [87]. Other sulfated fucans from genera of brown sea weeds *Dictyota mertensii, Lobophora variegata, Fucus vesiculosus* and *Spataglossum schroederi* successfully blocked reverse transcriptase activity in HIV infection [88]. The most prominent broad spectrum antiviral agents of algal origin are the lectins Cyanovirin and Griffithsin from *Nostoc* (a cyanobacterium) and *Griffithsia* (red alga) species respectively [88].

Laminarin: Laminaran is a glucan abundant in some species of brown algae like *Saccharina longicruiris* and *Ascophyllum nodosum*, an effective inhibitors of HIV replication and proliferation. The mechanism of action is via prevention of adsorption of HIV onto human-derived lymphocytes and HIV reverse transcriptase [87].

Naviculan: This is a sulfated polysaccharide from the diatom *Navicula directa* with activity against HSV-1 and HSV-2 at a low IC_{50} value <14 μg/ml and the influenza virus by inhibiting the initial stages of viral replication and potentially blocking viral internationalization into host cells [89].

Calcium Spirulan: Another polysaccharide extracted from the marine blue-green algae (Cyanobacteria) *Arthrospiroa (Spirulina) platensis* capable of inhibiting HSV-1 (in HeLa cells); HCMV (in HEL cells), influenza A (in MDCK cells) [90].

4.1.3. Bacterial Polysaccharides

The polysaccharide calcium spirulan extracted from *Arthrospira platensis* showed broad spectrum activity against the following viruses: HSV-1, human

cytomegalovirus (HCMV), measles virus, mumps virus, influenza A and HIV-1 by preventing viral penetration into host cells. While nostoflan isolated from *Nosctoc flagelliforme* (a cyanobacterium) prevented adsorption of the same viruses (HSV-1; HSV-2; HCMV and influenza virus A at maximum inhibitory doses of 0.37, 2.9, 0.47 and 78 μg/mL respectively [91]. Exopolysaccharides of other bacteria species like *Streptococcus thermophilus* had another mechanism of action against viruses by activating the Toll-like receptor S and expression of interferons in porcine intestinal epithelial cell that are responsible for innate antiviral immune response [92].

4.2. Fungal Polysaccharides

High molecular weight polysaccharides extracted from *Porodaedalea pini* are capable of reducing plaque formation by herpes simplex virus 1 (HSV-1) at an effective dose of 5 g/mL in Vero cells. Another polysaccharides (EP-AV2) with a lower molecular weight reduced plaque formation the coxsackie virus B3 (CVB3) in HeLa cells at an effective dose of 1 mg/mL [93]. Protein-bound polysaccharides from *Ganoderma lucidum* a medicinal mushroom were active against HSV-1 and HSV-2 [94]. The acidic protein-bound polysaccharide was more reactive than the neutral one at EC_{50} concentrations <100 μg/ml [94]. Lentinan a polysaccharide from *Lentinus edodes* inhibited the infectious hematopoietic necrosis virus (IHNV) by immediate inactivation and viral replication limitation [95]. Acidic polysaccharides from *Cordyceps militaris* reduced the viral titer of influenza virus (H1N1) in the bronchoalveolar area and lung of mice while increasing the TNF-α and interferon (IFN) levels in the blood [96]. *Agaricus blazei* polysaccharide (ABP-AW1) reduced CPE of Western equine encephalitis virus while sulfated polysaccharides from *Agaricus brasiliensis* inhibited HSV-1 and acyclovir-resistant strains 29R [97]. Tyrosinases-proteins isolated from *Agaricus bisporus* showed antiviral activity against Hepatitis C virus by catalyzing selective hydroxylation of crucial position tyrosine residues in viral proteases [98]. GLPG a proteoglycan isolated from *Ganoderma lucidum* inhibited Herpes simplex virus types 1 and 2 in a dose-dependent manner with no cytotoxic effect even at high concentrations (2000μg/ml). Polysaccharide (AHCC) extracted from *Lentinus edodes* mycelium showed broad-spectrum antiviral activity inhibiting West Nile Virus, influenza virus, hepatitis virus and human papillomavirus [99] [100]. Lentinan (LNT-1) extracted from *Lentinus edodes* inhibited IHNV infection progression in cells by direct inactivation and inhibition of viral replication [101].

4.3. Plant Polysaccharides

Polysaccharides of the plant *Chuaminshen violaceum* were active against Newcastle disease virus (NDV) by inhibition of viral adsorption and penetration into cells [102]. A mixture of five highly branch polysaccharide-protein conjuncts (LbGp1-5) extracted from *Lycium barbarum* showed antiviral and immunomo-

dulatory effects on H1N1-induced viral pneumonia in *in vivo* and *in vitro* tests by blocking viral attachment and entry, reducing viral load in lung, regulating the phenotype of pulmonary macrophages and inhibiting excessive inflammation [103]. Another sulfated polysaccharide from the plant *Caesalpinia ferrea* showed effects against Herpes simplex virus (HSV) and Poliovirus (PV) by inhibiting virus adsorption, and synthesis of viral proteins and had the promising effect of low cellular toxicity [104]. *Achyranthes bidentata* polysaccharide sulfate (ABPS) isolated from showed activity against porcine reproductive and respiratory syndrome virus (PRRSV) at low concentrations [105]. Other polysaccharides extracted from the plant *Portulaca aleracea* showed inhibitory effects against Herpes simplex virus type 2 (HSV-2) and influenza virus a (IFV-A) with the pectic polysaccharides outperforming the acidic and neutral polysaccharides. The drug PG2® lyophilized injection is an injectable product containing an active fraction of polysaccharide which was extracted, and purified from the roots of *Astragalus membranaceus* that has been used for the treatment of COVID-19 with success [106]. It is now known that PG2 can upregulate microRNAs having antiviral potential and anti-inflammatory ability of macrophages, thereby driving M2 macrophage polarization and improving the inflammatory lung microenvironment [107]. In 2019, our lab demonstrated that certain components of *Zanthoxylum armatum* and *Hibiscus sabdariffa* show antiviral effects, especially against the norovirus [100].

Animals: There are more than eleven animal toxins such as the cone snail peptide (ziconotide), two lizards' peptides (exenatide and lixisenatide) two leech peptides (bivalirudin and desirudin) and six snake venom-related drugs (captopril, cabrotide, enalapril, eptifibatide, tirofiban and batroxobin) has officially been sold as drugs in the market [101].

Scorpion: There are studies that document the antibacterial, anticancer, and antiviral properties of these small peptides isolated from scorpion venom [102]. There are numerous antiviral scorpion peptides classified as small molecule NDBPs that have been identified such as Ctry2459, Hp1090 and mucroorin-M1 [103].

The scorpion peptide -Ctry2459 extracted from *Chaerilus tryznai* has been reported to be active against HCV by inactivating infectious viral particles, but the toxin Hp1090 isolated from *Heterometrus petersii* caused direct inhibition by permeating the viral phospholipid membrane [104] [105]. Another histidine rich peptide -Eval418 isolated from *Euscorpiops valifus* had broad spectrum effect on HCV and HSV-1 [104] [105]. The mucroporinM1 showed remarkable broad-spectrum activity against measles, SARS-CoV, influenza, H5N1 virus and HBV infections [103]. Scorpion venom compounds have been reported to have the ability of their disulfide-bridged peptides (DBPs) to bind to HIV gp 120 glycoprotein because they mimic lentiviruses host cell $CD4^+$ receptor cells [106]. They can therefore stop the gp-CD4 interaction, an essential component with the capacity viral entry to host cells [57].

Snake: Snake venom has also been implicated in antiviral activities specifically against measles, Sendai virus, dengue virus, yellow fever virus and HIV and been demonstrated in clinical trials [107]. The antiretroviral activity of snake venom was attributed to effect on HIV-1 glycoprotein or protease with the ability to decrease viral load and T $CD4^+$ cell count [107]. Novel studies reveal that snake Phospholipase A2, (PLA2s) function as antiviral agents by inhibiting syncytium formation between HIV-infected cells and healthy $CD4^+$ cells, preventing HIV from binding to cells. The study also showed that only (Phospholipase A2) PLA2s that are dimeric have virucidal effects and they do this by destruction of the viral membrane [108].

5. Conclusion

The number of natural products with antiviral activity shown in this review are numerous ranging from parent plants to animal parents. Record show that polytherapy is effective strategy used in the treatment of viral infections. As has been adjudged in this review, natural products inherently use the combinational approach as their mechanism of action because of the myriad of compounds they possess and potential reactivity of these molecules. Broad-spectrum antiviral agents could be produced and the problem of antiviral drug resistance stalled. Sources like snake and scorpion venom should not also be avoided as they potentially have bioactive compounds to be harnessed. Marine fungi and algae are another grossly underutilized resource with hidden treasures. Polysaccharides from algae, bacteria, fungi and plants have been ignored, but record show their broad-spectrum antiviral activity. Polysaccharides and other compounds with natural parent need attenuation be more reactive and attain broad-spectrum status, these should also be investigated. The new innovations like monoclonal antibodies should also be embraced, increasing the number of antivirals while establishing broad-spectrum antivirals of natural origin.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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