Natural Products-Derived Chemicals: Breaking Barriers to Novel Anti-HSV Drug Development

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Abstract: Recently, the problem of viral infection, particularly the infection with herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), has dramatically increased and caused a significant challenge to public health due to the rising problem of drug resistance. The antitherpetic drug resistance crisis has been attributed to the overuse of these medications, as well as the lack of new drug development by the pharmaceutical industry due to reduced economic inducements and challenging regulatory requirements. Therefore, the development of novel antiviral drugs against HSV infections would be a step forward in improving global combat against these infections. The incorporation of biologically active natural products into anti-HSV drug development at the clinical level has gained limited attention to date. Thus, the search for new drugs from natural products that could enter clinical practice with lessened resistance, less undesirable effects, and various mechanisms of action is greatly needed to break the barriers to novel antitherpetic drug development, which, in turn, will pave the road towards the efficient and safe treatment of HSV infections. In this review, we aim to provide an up-to-date overview of the recent advances in natural antitherpetic agents. Additionally, this paper covers a large scale of phenolic compounds, alkaloids, terpenoids, polysaccharides, peptides, and other miscellaneous compounds derived from various sources of natural origin (plants, marine organisms, microbial sources, lichen species, insects, and mushrooms) with promising activities against HSV infections; these are in vitro and in vivo studies. This work also highlights bioactive natural products that could be used as templates for the further development of anti-HSV drugs at both animal and clinical levels, along with the potential mechanisms by which these compounds induce anti-HSV properties. Future insights into the development of these molecules as safe and effective natural anti-HSV drugs are also debated.

Keywords: herpes simplex virus infection; bioactive natural products; drug resistance; drug development; antitherpetic drugs; preclinical and clinical studies; mechanisms of action
1. Introduction

Infection with herpes simplex virus (HSV) has been recognized since antiquity in humans, however, the first in vitro cultivation of HSV was assayed in 1925 [1]. Since 1968, herpes simplex type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) have been distinguished from each other by different clinical manifestations and tropism [2]. HSV belongs to Herpesviridae, which is a broad family of enveloped-DNA viruses that induce numerous clinically substantial syndromes in both adults and neonates. Several factors including viral entrance, the nature of the disease, and degree of host immune competency could affect the induced syndromes [3,4]. HSV-1 is generally associated with oral or facial infection and encephalitis, while HSV-2 is accountable for genital herpes, which is an important sexually transmitted disease [5]. Moreover, infection with HSV-2 can cause recurrent, painful genital lesions and is often connected with negative psychosocial consequences such as shame, anxiety, and depression. Furthermore, infection with HSV-2 was observed to be a high-risk factor for potential HIV infection, as well as invasive cervical carcinoma [6]. Several reports have declared that HSV is involved in various ocular diseases, including stromal keratitis, endothelitis and neurotrophic keratopathy [4–7]. The current existant treatment of HSV infection relies mainly on the use of acyclovir (ACV) and related synthetic nucleoside analogs. Unfortunately, the rigorous utilization of these drugs has led to the establishment of undesirable effects as well as drug-resistant strains [8,9]. Although imperative efforts were taken to develop a vaccination, no vaccines have been validated or marketed for effective prevention of the infection to date. Therefore, the development of new antiviral medications has earned much attention in recent decades [10,11]. While many anti-HSV drugs have already been developed and engaged in the treatment of HSV infections, the search for different sources of anti-HSV drugs is a great task for many researchers and healthcare providers to conquer challenges with drug resistance [12]. Thus, it is an important concern to open new gates to search for new therapeutic agents that perform with different mechanisms of action than nucleoside analogs. Nature is a very rich source of these molecules.

2. Epidemiology and Pathogenesis of HSV Infection

It’s acknowledged that HSV endures for the lifetime of the host in the form of latent infection in the peripheral neurons [13]. After infection begins, reactivation can be systematically triggered by re-entering the lytic phase of replication to create a progeny virus for spreading [14]. However, during latent infection, the viral lytic genes are largely down-regulated, and their promoters are joined into repressive heterochromatin (Figure 1). Consequently, reactivation necessitates viral lytic gene expression to be created by silenced promoters in the absence of viral proteins [15]. During primary infection, HSV penetrates through breaks in the skin or mucosa and subsequently attaches to and accesses epithelial cells and starts replication. It’s taken up by free sensory nerve endings placed at the dermis, and the nucleocapsid containing the viral genome is transferred by retrograde axonal flow to the nucleus in the sensory ganglion [16,17]. Skin symptoms include vesicular lesions on an erythematous base. Lesions drive to the focal damage of the epithelial layer and a widespread infiltrate of inflammatory cells elaborates in the surrounding rim and the underlying dermal layer [15,18]. It has been estimated that 10–30% of new infections are symptomatic. After recovering from the initial infection, HSV perseverance latently in the sensory ganglion for the life of the host. Regularly, the virus reactivates from the latent state and moves back down the sensory nerves to the skin or mucosal surface [15,19]. Viral shedding can appear either in the presence of lesions as a clinical reactivation or with very moderate or no symptoms as subclinical reactivation. Shedding from mucosal surfaces drives transmission to other sexual partners and, in some cases, infection with HSV can be transferred from mother to infant at delivery [20,21].
Figure 1. A graphical illustration shows the epidemiology and pathogenesis of herpes simplex virus (HSV) infection. Detailed descriptions are discussed in Section 2.

3. Natural Products-Derived Molecules with Anti-HSV-1 and Anti-HSV-2 Properties

As a part of our ongoing search for natural compounds that are effective against HSV infection, we tried to evaluate progress by reviewing the compounds showing promising anti-herpetic activities. The reviews of the literature covering this area were published previously [22–24], with the latest in 2015 [25]. Thus, we followed the latest information and gathered approximately 83 literature sources, which were not included in these papers, showing natural compounds with anti-HSV activity. The SciFinder database was used to cover this area of the published literature and selected data for compounds obtained by the search are presented in Tables 1–6 and Figures 2–5.
Table 1. The overview of phenolic compounds with anti-HSV activity.

| Compound | Antitherpetic and Cytotoxicity Assays, Strains, Cells, and Reference Agents | Results | Additional Information | Source |
|----------|---------------------------------------------------------------------------|---------|------------------------|--------|
| Epicatechin (1) | MTT cell viability ACV SI = 4.5 | HSV-1, SI = 6.0 (29-R) | | |
| Epigallocatechin (2) | | HSV-1, SI = 5.2 (KOS), 12.8 (29-R) | Dietary phenolics | [26] |
| Robinetinidol(4α-6)gallocatechin (3) | | HSV-1 SI = 5.2 (KOS), 5.0 (29-R) | | |
| Apigenin (4) | | HSV-1 EC50 = 5 μM, SI = 50; HSV-2 EC50 N/A, SI N/A | | |
| Baicalin (5) | | HSV-1 EC50 = 5 μM, SI= 200; HSV-2 EC50 N/A, SI N/A | | |
| Catechin hydrate (6) | | HSV-1 EC50 = 4 μM, SI = 250; HSV-2, EC50 N/A, SI N/A | | |
| Chrysin (7) | | HSV-1 EC50 = 2.5 μM, SI= 4; HSV-2 EC50 N/A, SI N/A | | |
| Flavonoids | Epicatechin (1) | | Dietary phenolics, green tea, propolis, some flavonoid rich medicinal plants. Flavanols and flavonols appear to be more active than flavones. Furthermore, treatment of Vero cells with ECG (8) and galangin (11) before virus adsorption led to a slight enhancement of inhibition, indicating that an intracellular effect may be involved. | [27] |
| | Epicatechin gallate (8) | CPE, PRA, YRA ACV for HSV-1 EC50 = 50 μM, SI = 10; HSV-2, EC50 = 50 μM, SI = 10 | | |
| | Epigallocatechin (2) | HSV-1, EC50 = 2.5 μM, SI = 100; HSV-2, EC50 N/A, SI N/A | | |
| | Epigallocatechin gallate (9) | HSV-1, EC50 = 2.5 μM, SI = 40; HSV-2, EC50 N/A, SI N/A | | |
| | Fisetin (10) | HSV-1 EC50 2.5 μM, SI = 40; HSV-2 EC50 N/A, SI N/A | | |
| | Galangin (11) | HSV-1 EC50 2.5 μM, SI = 400; HSV-2 EC50 N/A, SI N/A | | |
| | Genistein (12) | HSV-1 EC50 5 μM, SI = 50; HSV-2 EC50 50 μM, SI = 5 | | |
| | Kaempferol (13) | HSV-1 EC50 15 μM, SI = 3.3; HSV-2 EC50 N/A, SI N/A | | |
| Quercetin (19) | Raw 264.7 and Vero cells, HSV-1 PRA, Western blot analysis, quantitative RT-PCR | Reduction in plaque formation of 90% at 30 μg/mL | Inhibition of the expressions of HSV proteins (gD, ICP0) and genes (ICP0, UL13, UL52). Specific suppression of the expression of TLR-3, inhibition of transcriptional factors NF-κB and IRF3. |
|-----------------|-------------------------------|---------------------------------|-----------------------------------------------|
| **Epigallocatechin gallate (9)** | IP (%) | IP: 100% | Dietary phenolic, green tea component |
| | % PFU | At 1 μM cca 40%, at 5 μM cca 5% | [29] |
| **Houttuynoid M (20)** | PFA ACV IC₅₀ 0.15 μM; SD 1333 | IC₅₀ 17.72 μM; SI> 11.29 IC₅₀ 12.42 μM; SI> 16.10 | Houttuynia cordata [31] |
| **Houttuynoid A (21)** | 1. β-galactosidase assay - the activity of enzyme measured in cell lysates 2. PRA 3. Progeny HSV-1 yield assay - effect on HSV-1 multiplication | 1. HSV-1 (F) IC₅₀ 23.50 ± 1.82 μM, CC₅₀ 166.36 ± 9.27 μM 2. HSV-1 (F) IC₅₀ of 21.08 μM 3. HSV-1 (F) multiplication reduced by 100% at 75 μM | Possible mechanism—blocking viral membrane fusion [32] |
| **Genistein (12)** | Vero cells, HSV-1 (KOS), HSV-1 (29 R), HSV-2 (333) PRA | IC₅₀ (μM); SI: HSV-1 (KOS)/HSV-1 (29 R)/HSV-2 (333) 14.02, 3.88/7.76, 7.01/14.12, 6.95 | Isoflavonoid, soya beans, alfalfa [33] |
| Compound          | Cell Line | Virus | EC50/IC50 (μg/mL) | SI | Notes |
|------------------|-----------|-------|-------------------|----|-------|
| Kuwanon C (22)   | Vero cells, HSV-1 (KOS, VR733) | CPE as 50% tissue culture infective dose (TCID50/50 μL) | Reduced the titer by 2.9 log<sub>10</sub> against strain KOS and by 3.18 log<sub>10</sub> against strain VR733 | | [35] |
| Kuwanon T (23)   | Vero cells, HSV-1 | ACV IC<sub>50</sub> 1.45 μg/mL; SI 144.8 | | | |
| Kuwanon U (24)   | Vero cells, HSV-1 | ACV IC<sub>50</sub> 1.65 μg/mL; SI 127.3 | | In silico analysis along with antibacterial and anti-inflammatory effects [34] | |
| Kuwanon E (25)   | Vero cells, HSV-1 (KOS, VR733) | | | | |
| Luteoforol (26)  | Vero cells, HSV-1 (KOS, VR733) | | | | |
| Luteolin (14)    | Vero cells, HSV-2 | AcV EC<sub>50</sub> 2.6 μg/mL, SI= 42.53 | | Dietary flavonoid [36] | |
| Theaflavin-3,3′-digallate (27) | Vero cells, HSV-1 | EC<sub>50</sub> 20 μM; SI = 5.625 | | Green tea [37] |
| Compound                          | Source Cells, Viral Strains | IC₅₀/CC₅₀ Values | Activities and Sources |
|----------------------------------|-----------------------------|-----------------|------------------------|
| Cycloartocarpin (28)             | Vero cells, HSV-1 (KOS), HSV-2 (186) PRA ACV | HSV-1 IC₅₀ 28.2 μM; HSV-2 IC₅₀ 23.5 μM | Prenylated phenolics Morus spp., Artocarpus spp. [38] |
| Isocyclomorusin (29)             | HSV-1 IC₅₀ 30.4 μM; HSV-2 IC₅₀ 27.2 μM | | |
| Norartocarpin (30)               | HSV-1 IC₅₀ 63 μM; HSV-2 IC₅₀ 52.2 μM | | |
| Catechin-7-gallate (31)          | Vero cells, HSV-1 (KOS), HSV-2 (186) PRA ACV | CC₅₀ 43.2 ± 2.3 μg/mL | Dietary phenols Low activity, questionable results [39] |
| Kaempferol-3-O-6’-O-galloyl-β-D-glucopyranoside (32) | ACV CC₅₀ >200 ± 0.4 μg/mL | CC₅₀ 124.1 ± 1.2 μg/mL | Dietary phenols Low activity, questionable results [39] |
| Kaempferol (13)                  | Vero cells, HSV-1 CPE | CC₅₀ 76.1 ± 0.2 μg/mL | Dietary phenols Low activity, questionable results [39] |
| Quercetin-3-O-6’-O-galloyl-β-D-glucopyranoside (33) | ACV CC₅₀ >200 ± 0.4 μg/mL | CC₅₀ 175.6 ± 0.9 μg/mL | Dietary phenols Low activity, questionable results [39] |
| Quercetin (19)                   | Vero cells, HSV-1 CPE | CC₅₀ 78.1 ± 0.8 μg/mL | Dietary phenols Low activity, questionable results [39] |
| 7-O-galloylitrictiflavan (34)    | Vero cells, HSV-1 (KOS), HSV-2 (333) PRA | IC₅₀ 30 μg/mL | Pithecellobium clypearia Other viruses tested [40] |
| 7,4’-di-O-galloylitrictiflavan (35) | Vero cells, HSV-1 (KOS), HSV-2 (333) PRA | IC₅₀ 20 μg/mL | Other viruses tested [40] |
| Strychnobiflavone (36)           | Vero cells, HSV-1 (KOS), HSV-2 (333) PRA | HSV-1 (KOS) IC₅₀ 11.82 μg/mL, SI = 22.61; HSV-2 (strain 333) IC₅₀ 6.31 μg/mL, SI = 42.33 | Strychnos pseudoquina [41] |
| Ethyl 2,4-dihydroxybenzoate (37) | Vero cells, HSV-1 (KOS), HSV-2 (333) PRA | HSV-1 IC₅₀ 1.32 ± 0.44 μg/mL; SI 159.1 | In silico analysis; antibacterial and anti-inflammatory effects [34] |
| Gallic acid (38)                 | Vero cells, HSV-1 CPE | CC₅₀ 49.8 ± 0.4 μg/mL | Dietary phenols Low activity, questionable results [39] |
| Alkyl derivatives of gallic acid | Octyl gallate (39) | IP (%) | IP: 100 % | Dietary phenolics | [29] |
|--------------------------------|-------------------|--------|-----------|-------------------|-----|
|                                | HEp-2 and Vero cells, HSV-1 CPE | Octyl gallate directly inactivates HSV-1 (virucidal activity). 39 suppresses both the intracellular multiplication and the release of the virus. 39 selectively accelerates the death of the virus-infected cells. The addition of the compound (39), even at 6 h post-infection, completely abolished the formation of progeny virus in the infected cells. | Other viruses tested including HSV-1: Inhibition was enhanced by the compounds with a higher number of carbons in the alkyl moieties, maximum at 12 (lauryl gallate), however, cytotoxicity was increased. | | |
| Chebulagic acid (40) | AV | IPF | ACV IC₅₀ 29.04 ± 1.04 µg/mL | HSV-2 IC₅₀ 1.41 ± 0.51 µg/mL | Dose-dependently potent in vitro direct anti-viral activity. Effective prevention of the attachment as well as penetration of the HSV-2 to Vero cells. | [42] |
| Chebulinic acid (41) | IPF | | HSV-2 IC₅₀ 0.06 ± 0.002 µg/mL | | | |
| Tellimagrandin I (42) | IPF | At 0.75 µg/mL ACV completely protected Vero cells against infection | EC₅₀ of 2.6 µM for the direct mode, 5.0 µM for the absorption mode. | Ellagitannin—Corrus spp., Eucalyptus spp., Melaleuca styphelioides | [43] |
| N-trans-ferulolyl tyramine (43) | Vero cells, HSV-2 | EC₅₀ 0.92 µg/mL, SI = 217 | | Dietary phenolic, metabolite of gut degradation of phenolics | [45] |
| Protocatechuic acid (44) | Vero cells, HSV-1, HSV-2 | AV EC₅₀ 1.43 µg/mL, SI= 140 | EC₅₀ 0.92 µg/mL, SI = 217 | Dietary phenolic, metabolite of gut degradation of phenolics | [45] |
| Psoromic acid (45) | Vero cells, HSV-1, HSV-2 | AV for HSV-1 IC₅₀ 2.6 µM; SI 119.2; for HSV-2 EC₅₀ 2.8 µM; SI 110.7 | HSV-1 IC₅₀ 1.9 µM; SI 163.2 | Study of synergy with ACV and inhibition of HSV-1 DNA polymerase (in vitro and in silico assays). | [46] |
| Rhinacanthinic acid C (46) | Vero cells, HSV-2 PRA | ED₅₀ 58.98 µg/mL | | Rhinacanthus nasutus | [47] |
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| **Coniferyl aldehyde (54)** | Vero cells, HSV-1, HSV-2 (KOS), HSV-1 (29 R), HSV-2 (333) PRA | IC₅₀ (μM), SI: HSV-1 (KOS)/HSV-1 (29 R)/HSV-2 (333) 11.62, 9.6/3.34, 31.52/35.53, 28.14 | Phenolic, *Quercus suber, Simira glaziovii, S. eleiezeriana* | [51] |
| **Coumestrol (55)** | Vero cells, HSV-1 (KOS), HSV-1 (29 R), HSV-2 (333) PRA | ACV: IC₅₀ 2.44 μM, SI >1818 (KOS), NA (29 R), | Coumestan, soya beans, alfalfa | [33] |
| **Kuwanon X (51)** | Vero cells, HSV-1 (15577 and clinical strains), HSV-2 (333) PRA | ACV IC₅₀ 0.1 μg/mL for all strains | HSV-1 IC₅₀ 2.2 and 1.5 μg/mL; HSV-2 IC₅₀ 2.5 μg/mL | [50] |
| **Mulberrofuran B (52)** | Vero cells, HSV-2 TRA ACV IC₅₀ 1.65 μg/mL; SI 127.3 | HSV-2 IC₅₀ 0.93 ± 0.23 μg/mL; SI 225.8 | *In silico* analysis; antibacterial and anti-inflammatory effects | [34] |
| **Oxyresveratrol (53)** | Vero cells, HSV-1 (KOS), HSV-2 (186) PRA ACV HSV-1 IC₅₀ 1.5 μM; HSV-2 IC₅₀ 2.9 μM | HSV-1 IC₅₀ 42.8 μM; HSV-2 IC₅₀ 42.5 μM | Stilbenoid *Morus spp., Artocarpus spp.* | [38] |
| **Stilbenoids and 2-arylbenzofurans** | AcV EC₅₀ 3.0 μM | HSV-1 EC₅₀ 6.25 μM | 5.5 μg/mL, SI 44.8 | [48] |
| **Aspergilol H (48)** | HSV-1 | HSV-1 EC₅₀ 4.68 μM | HSV-1 EC₅₀ 6.25 μM | Deep-sea fungus *Aspergillus versicolor* | [49] |
| **Aspergilol I (49)** | HSV-1 | HSV-1 EC₅₀ 3.12 μM | HSV-1 EC₅₀ 6.25 μM | AcV: IC₅₀ 5.8 μg/mL, SI = 18.97, HSV-2 IC₅₀ 5.5 μg/mL, SI = 20.0 | *Antrodia camphorate* Additive effect of 47 with ACV | [48] |
| **Coccoquinone A (50)** | Vero cells, HSV-1, HSV-2 PRA ACV HSV-1 IC₅₀ 2.1 μg/mL, SI = 61.9, HSV-2 IC₅₀ 2.9 μg/mL, SI = 44.8 | HSV-1 IC₅₀ 5.8 μg/mL, SI = 18.97, HSV-2 IC₅₀ 5.5 μg/mL, SI = 20.0 | HSV-1 EC₅₀ 6.25 μM, SI 42.8 μM, SI | *Antrodia camphorate* Additive effect of 47 with ACV | [48] |
| **Anthrones** |  |  |  | Preylated phenol, *Morus spp.* 51 did not inactivate cell-free HSV-1 particles but inhibited cellular adsorption and penetration of HSV-1 viral particles. Following viral penetration, 51 reduced the expression of HSV-1 IE and L genes and decreased the synthesis of HSV-1 DNA. Furthermore, 51 inhibited the HSV-1-induced nuclear factor (NF)-κB activation through blocking the nuclear translocation and DNA binding of NF-κB. | [50] |

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| **Curcumin** (56) | CPA, PRA, viral adsorption assay, viral penetration assay | At 30 μM, 85% inhibition of HSV-1 and 68% of HSV-2 CPE, PRA 92% for HSV-1 and 88% for HSV-2 | *Curcuma longa* Inhibits HSV adsorption and replication [52] |
|-------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| Vero cells, HSV-1 CPE | ACV CC₅₀ > 200 ± 0.4 μg/mL | CC₅₀ 49.8 ± 0.4 μg/mL | Dietary phenols Low activity, questionable results [39] |
| **Imperatorin** (57) | ACV – full inhibition of replication of HSV-1 at 250 μg/mL | 57 decreases titer of HSV-1 by 55.6% at 31.25 μg/mL | Furanocoumarin of Apiaceae family [53] |

| Pinoresinol (58) | IP (%) | IP: 26% | Dietary phenolics [29] |

HSV-1: herpes simplex virus type 1; HSV-2: herpes simplex virus type 2; ACV: acyclovir; CPE: cytopathic effect; IC₅₀: 50% inhibitory concentration; EC₅₀: 50% effective concentration; ED₅₀: 50% effective dose; CC₅₀: 50% cytotoxic concentration; PRA: plague reduction assay; YRA: yield reduction assay; SI: selectivity index = CC₅₀/EC₅₀ or CC₅₀/IC₅₀ (cytotox./antiviral); PFU: plaque forming units; IPF: inhibition of plaque formation; TRA: titer reduction assay; MTT assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide; Vero cells used for assay if not stated in methods; F, KOS, 29-R—viral strains.
Figure 2. Phenolic compounds with antiherpetic activity.
Table 2. The overview of alkaloids with anti-HSV activity.

| Compound          | Antiherpetic and Cytotoxicity Assays, Strains, Cells, and Reference Agents                                                                 | Results                                                                 | Additional Information                                                                 | Source |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|--------|
| Harmine (59)      | Vero cells, HSV-2 PRA ACV CC\(_50\) and IC\(_50\) > 3.000 mg/mL and 0.1 μg/mL, respectively, SI > 30.000                             | CC\(_50\) and IC\(_50\) 12.5 μg/mL and 0.3 μg/mL, respectively, SI = 41.6 | *Peganum harmala, Banisteriopsis caapi, Passiflora incarnata*                                                                 | [54]    |
|                   | Human foreskin fibroblasts (HFF), HSV-1 (166vVP22-GFP) GFP-based reporter assay Cidofovir at 3 μM reduced to 20%                                         | At 3.3 μM, 59 reduced HSV-1 replication to approx. 50%, at 10 μM to approx. 5% | 59 inhibited viral protein expressed as a dual-specificity tyrosine phosphorylation-regulated kinase inhibitor. | [55]    |
| Harmane (60)      | Vero cells, HSV-1, HSV-2 PRA ACV HSV-1 EC\(_50\) 0.8 μg/mL                                                                 | HSV-1 EC\(_50\) 4.9 μg/mL, SI = 11.8                                     | *P. harmala, B. caapi, P. incarnata*                                                                                     | [51]    |
| Aaptamine (61)    | Vero cells, HSV-1 CPE                                                                                                                | EC\(_50\) 7.0 μg/mL                                                     | Marine sponge *Aaptos* spp.                                                                                              | [56]    |

Figure 3. Alkaloids with antiherpetic activity.
Table 3. The overview of terpenoid compounds with anti-HSV activity.

| Compound | Antitherpetic and Cytotoxicity Assays, Strains, Cells, and Reference Agents | Results | Additional Information | Source |
|----------|-----------------------------------------------------------------------------|---------|------------------------|--------|
| **Monoterpenes** | | | | |
| Geraniol (62) | Vero cells, HSV-2 ACV (EC\(_{50}\) 1.94 μg/mL; SI = 108.25) | HSV-2 EC\(_{50}\) 1.92 μg/mL SI = 109.38 | *Thymus bovei* Benth. essential oil, typical monoterpenes of Lamiaceae | [57] |
| Cypellocarpin C (63) | Vero cells, HSV-1 (KOS), HSV-2 (clinical isolates) PRA, TRI ACV HSV-1 IC\(_{50}\) 1.92 ± 0.23 μg/mL, SI > 109.4, HSV-2 IC\(_{50}\) 1.75 ± 0.33, SI > 120.0 | HSV-1 IC\(_{50}\) 0.96 ± 0.12, SI > 218.8 | 63 is a cross-metabolite of monoterpenic glycoside and a methylchromone, *Eucalyptus globulus* | [58] |
| (+)-rhodonoid C (64) | Vero cells, HSV-1 CPE ACV IC\(_{50}\) 4.2 μM, SI > 100 | IC\(_{50}\) 80.6 ± 4.7 μM, SI = 2.7 | 64 is a cross-metabolite of monoterpenes and polyketide *Rhododendron* spp. | [59] |
| **Sesquiterpenes** | | | | |
| β-caryophyllene (65) | Vero cells, HSV-1, HSV-2 (clinical isolates), (HSV-2 ACV-resistant) PRA Time-of-addition assay Virus inactivation assay ACV HS2-2 IC\(_{50}\) 0.14 μg/mL; SI = 1178; HSV-2 (acyclovir-resistant) EC\(_{50}\) 71.84 μg/mL, SI = 2.29 | HSV-2 EC\(_{50}\) 5.38 μg/mL SI = 9.10 HSV-2 (acyclovir resistant) EC\(_{50}\) 5.02 μg/mL SI > 9.76 | Bicyclic sesquiterpene, common occurrence, for example, cloves | [60] |
| Kellerin (66) | Vero cells, HSV-1 (KOS) PRA ACV at 2.5 μg/mL, 82% of plaque reduction 66 at 2.5 μg/mL 65% | | 66 is a cross-metabolite of sesquiterpene and coumarin Gum resin of *Ferula assa foetida* No cytotoxic effect up to 10 μg/mL | [61] |
| Lactarorufin A 8-[N-benzoyl-(2'R,3'S)-3'-phenylisoserinate] (67) | Vero cells, HSV-1 (MacIntyre strain) | HSV-1 IC\(_{50}\) 17.3 μg/mL, SI = 16 | Taxol-N-benzoylphenyl-isoserinates of sesquiterpenoid alcohols and sesquiterpenoids | [62] |
| Compound                                      | CPE/EC50/IC50 | SI  | Source                                      |
|-----------------------------------------------|---------------|-----|---------------------------------------------|
| Isolactarorufin 8-[N-benzyol-(2'R,3'S)-3'-phenylisoserinate] (68) | ACV IC₅₀ 1 μg/mL, SI > 250 |     | *Lactarius* mushroom                         |
| Furandinol 8-[N-benzyol-(2'R,3'S)-3'-phenylisoserinate] (69)       | ACV IC₅₀ 1 μg/mL, SI = 17.4 |     |                                               |
| Isovetterol 13-[N-benzyol-(2'R,3'S)-3'-phenylisoserinate] (70)     | HSV-1 IC₅₀ 15 μg/mL, SI = 19.3 |     |                                               |
| 5-deoxylactarolid B 8-[N-benzyol-(2'R,3'S)-3'-phenylisoserinate] (71) | HSV-1 IC₅₀ 7.8 μg/mL, SI = 13.9 |     |                                               |
| Isolactarorufin 8-epi-[N-benzyol-(2'R,3'S)-3'-phenylisoserinate] (72) | HSV-1 IC₅₀ 4.2 μg/mL, SI = 18.4 |     |                                               |
| Alantolactone (73)                                           | Vero cells, HSV-1 CPE |     | Sesquiterpene *Inula helenium* [63]         |
| (-)-15-methoxy-3,6-peroxocupar-1-ene (74)                        | Vero cells, HSV-1 (KOS strain, VR-1493) PRA ACV at 2.5 μM 96.96% |     | Sesquiterpene *Schisandra sphenanthera* [64] |
| (R)-6,9-dihydroxy-1-oxo-14-noreudesm-5,7,9-triene (75)            | Vero cells, HSV-2 CPE inhibition method Quantitative PCR 2 log₁₀ reduction in HSV-2 yield at conc. 12.5 μM, IC₅₀ 6.25 μM |     | 14-Noreudesmane sesquiterpene *Elaeagnus rhamnoides* [65] |
| Simirane A (76)                                               | Vero cells, HSV-1, HSV-2 PRA ACV HSV-1 IC₅₀ 0.8 μg/mL |     | Erythroxylane diterpene, *Simira elizeeriana* [51] |
| Dodovisnoid D (77)                                            | Vero cells, HSV-1 CPE |     | Clerodane diterpenes *Dodonaea viscosa* [66] |
| Dodovisnoid F (78)                                             | ACV IC₅₀ 4.2 μM, SI > 100 |     |                                               |
| Atomaric acid (79)                                             | Vero cells, HSV-1 (ACR-29) CPE |     | Meroditerpenes from Brazilian seaweed *Stypopodium zonale* (DictyotaCl) [67] |
| Substance                        | Effect                                      |
|---------------------------------|---------------------------------------------|
| Epitaondiol (80)                | ACV IC₅₀ 1.2 μM, SI > 716.6                |
|                                 | EC₅₀ 1.34 μM, SI > 361.9                   |
| 10-deacetyl-baccatin III (81)   | Vero cells, HSV-1 (MacIntyre strain)        |
|                                 | CPE                                         |
|                                 | ACV IC₅₀ 1 μg/mL, SI > 250                 |
|                                 | HSV-1 IC₅₀ 52.7 μg/mL, SI > 9.5            |
|                                 | The activity may be associated with their influence on mitotic division. |
|                                 | [68]                                        |
| Andrographolide (82)            | Vero cells, HSV-1                          |
|                                 | PRA                                         |
|                                 | ACV IC₅₀ < 1 μg/mL                         |
|                                 | IC₅₀ 8.28 μg/mL                            |
|                                 | Ent-labdane diterpenes *Andrographis paniculata* No cytotoxic effect at virucidal concentration. |
|                                 | [69]                                        |
| Neoandrographolide (83)         | 14-deoxy-11,12-didehydroandrographolide (84)| |
| 10,18-diacetoxy-8-hydroxy-2,6-dolabelladiene (85) | Vero cells, HSV-1 |
|                                 | CPE                                         |
|                                 | ACV at 15 μM, 79% of CPE                   |
|                                 | At 50 μM, 89% of CPE                       |
|                                 | At 50 μM, 87% of CPE                       |
| 10-acetoxy-8,18-di-hydroxy-2,6-dolabelladiene (86) | Vero cells, HSV-1 |
|                                 | CPE                                         |
|                                 | ACV at 15 μM, 79% of CPE                   |
|                                 | At 50 μM, 89% of CPE                       |
|                                 | At 50 μM, 87% of CPE                       |
|                                 | Effect on HIV-1 reverse transcriptase.      |
|                                 | [70]                                        |
| Fomitopin D (87)                | Vero cells, HSV-1                          |
|                                 | Green fluorescent protein (GFP) expression |
|                                 | ACV IC₅₀ 2.18 μg/mL                       |
|                                 | HSV-1 IC₅₀ 17 μg/mL                       |
|                                 | Steroid                                    |
|                                 | Fungus *Fomitopsis*                        |
|                                 | [71]                                        |
| Lyonifoloside A (88)           | Vero cells, HSV-1 (F strain VR 733)         |
|                                 | CPE                                         |
|                                 | ACV EC₅₀ 0.41 μM, SI > 244                 |
|                                 | IC₅₀ 11.1 μM, SI = 2.1                     |
|                                 | EC₅₀ 3.7 μM, SI = 4.3                      |
|                                 | EC₅₀ 11.1 μM, SI = 5.2                     |
|                                 | 9,10-seco-cycloartanes 88–90, lanosterol derivatives 91–93 |
|                                 | *Lyonia ovalifolia*                        |
|                                 | [72]                                        |
| Lyonifolic acid A (89)         | Vero cells, HSV-1                          |
|                                 | CPE                                         |
|                                 | ACV EC₅₀ 0.41 μM, SI > 244                 |
|                                 | IC₅₀ 2.1 μM, SI = 7.6                      |
|                                 | EC₅₀ 6.4 μM, SI = 3.0                      |
|                                 | EC₅₀ 14.3 μM, SI > 7.0                     |
|                                 | Steroid                                    |
|                                 | Cucurbitane steroid                        |
|                                 | Cucurbitaceae                              |
|                                 | [73]                                        |
| Lyonoligeneric acid (90)       | Vero cells, HSV-1                          |
|                                 | CPE                                         |
|                                 | ACV EC₅₀ 1.74 μM, SI > 132.2               |
|                                 | IC₅₀ 0.94 μM, SI = 127.7                   |
|                                 | Cucurbitine steroid                        |
|                                 | Cucurbitaceae                              |
|                                 | [73]                                        |
| Lyonifolic acid C (91)         | Vero cells, HSV-1 (KOS)                     |
|                                 | PRA                                         |
|                                 | Acyclovir IC₅₀ 1.74 μM, SI > 132.2         |
|                                 | IC₅₀ 2.87 ± 0.78 μg/mL, SI = 15.53         |
|                                 | Cucurbitane steroid                        |
|                                 | Cucurbitaceae                              |
|                                 | [73]                                        |
| Lyonifoloside M (92)           | Vero cells, HSV-1 (KOS)                     |
|                                 | PRA                                         |
|                                 | Effects on HSV-1 attachment and penetration |
|                                 | IC₅₀ 5.63 ± 1.37 μg/mL, SI = 2.46          |
|                                 | Brazilian marine sponge *Petromica citrina* (Demospongiae) The observed anti-HSV-1 activity was found to be mediated by the inhibition of virus attachment and by the penetration into |
|                                 | [74]                                        |
| Lyonifoloside P (93)           | Vero cells, HSV-1 (KOS)                     |
|                                 | PRA                                         |
|                                 | Effects on HSV-1 attachment and penetration |
|                                 | IC₅₀ 5.63 ± 1.37 μg/mL, SI = 2.46          |
| Compound                        | Data/Results                                                                 | Notes                                                                 |
|--------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------|
| Halistanol sulfate C (97)      | ACV IC₅₀ 3.45 ± 0.42 μg/mL, SI > 580                                      | Vero cells, the virucidal effect on virus particles, and by the       |
|                                | IC₅₀ 6.09 ± 1.51 μg/mL, SI = 1.95                                        | impairment in levels of ICP27 and gD proteins of HSV-1.              |
|                                |                                                                            | Synergic effect with acyclovir                                       |
| Glycyrrhizic acid (98)         | HeLa cells, HSV-1 CPE                                                     |                                                                            |
|                                |                                                                            | At 1 and 2 mM, the inhibition ranged from about 78% to 85%           |
|                                |                                                                            |                                                                            |
| Glycyrrhetic acid (101) and its methylester (102) | Vero cells, HSV-1 strain (KOS) PRA ACV IC₅₀ 1.1 ± 0.09 μM, SI> 400 |                                                                            |
|                                |                                                                            | IC₅₀ 21.7 ± 0.06 and 8.1 ± 0.2 μM, respectively. SI = 3.9 and > 26, respectively. |
|                                |                                                                            |                                                                            |
| 3α-hydroxylup-20(29)-ene-23,28-dioic acid (99) | Vero cells, HSV-1 (15577) CPE ACV IC₅₀ 0.25 μg/mL, SI > 2000 |                                                                            |
|                                |                                                                            | IC₅₀ 31.3 μg/mL, SI = 3.8                                             |
| 3-epi-betulinic acid 3-O-sulphate (100) | Vero cells, HSV-1 TIC 0.14 mM |                                                                            |
|                                |                                                                            | IC₅₀ 20 μg/mL, SI = 5                                                  |
| Oleanolic acid (103)           | Vero cells, HSV-1 (strain F), HSV-2 (strain G) CPE Viral inactivation or virucidal assay |                                                                            |
|                                |                                                                            | HSV-1 EC₅₀ 6.8 ± 1.24 μg/mL, SI = 14.4                                |
|                                |                                                                            | HSV-2 EC₅₀ 7.8 ± 1.4 μg/mL, SI = 12.6                                |
|                                |                                                                            |                                                                            |
| Asprellanoside A (104)         | Vero cells, HSV-1 TIC 0.14 mM |                                                                            |
|                                |                                                                            | Sulphur containing triterpenoid saponins                             |

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[75] Halistanol sulfate C
[76] The hydroxylation at C-21 seems to be responsible for the reduction of anti-HSV-1 activity, the C-29 hydroxy group would eliminate the anti-HSV-1 activity. C-20 methoxy or carboxy groups should be responsible for the enhancement of activity.

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[77] 3α-hydroxylup-20(29)-ene-23,28-dioic acid

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[78] Possible inhibition of the early stage of HSV multiplication
| Substance                        | Source | IC\textsubscript{50} (μg/mL) | SI   | Literature |
|---------------------------------|--------|-----------------------------|------|------------|
| Oblonganoside H (105)           |        | PRA                         | TIC 0.18 mM |            |
|                                 |        | total inhibitory concentration (TIC) 0.0043 mM | |            |
| Tereticornate (106)             |        | PRA, TRI                    | HSV-1 IC\textsubscript{50} 0.96 ± 0.12, SI > 218.8, HSV-2 IC\textsubscript{50} 1.75 ± 0.33, SI > 120.0 | 106 - triterpene Eucalyptus globulus |
| Vero cells, HSV-1 (KOS), HSV-2 (clinical isolates) | | | | [58] |
| 3β-O-trans-ferulyl-20-hydroxy-lup-28-oic acid (107) | | PRA | HSV-1 IC\textsubscript{50} 0.71 ± 0.06, SI 5.2 | Triterpene Rhododendron latoucheae |
| Vero cells, HSV-1 (F strain VR 733) | | CPE inhibition method | | [80] |
Figure 4. Terpenoid compounds with antitherpetic activity.
Table 4. The overview of miscellaneous small molecules with anti-HSV activity.

| Compound | Antiherpetic and Cytotoxicity Assays, Strains, Cells, and Reference Agents | Results | Additional Information | Source |
|----------|---------------------------------------------------------------------------|--------|------------------------|--------|
| Trichobotrysin A (108) | Vero cells, HSV-1 PRA ACV IC₅₀ 3.50 μM | IC₅₀ 3.08 μM | Deep-sea-derived fungus *Trichobotrys effuse* | [81] |
| Trichobotrysin B (109) | Vero cells, HSV-1 PRA ACV IC₅₀ 3.50 μM | IC₅₀ 9.37 μM | Tetramic acid derivatives | |
| Trichobotrysin D (110) | Vero cells, HSV-1 (clinical isolate with >99% homology to isolate SK087 US4-6 genes), HSV-2 (clinical isolate >99% homology to isolate 99-62039 US4 gene) CPE, YRA Time-of-addition, adsorption inhibition, virucidal, penetration inhibition assays Macromolecular synthesis inhibition analysis ACV HSV-1 EC₅₀ 0.9 μg/mL; SI > 1000 / HSV-2 EC₅₀ 0.7 μg/mL; SI > 1000 | IC₅₀ 3.12 μM | |
| (L)-2-(2,4-hexadiynyliden)-1,6-dioxaspiro[4.5]dec-3-ene (111) | | | **Tanacetum vulgare** Spiroketal-enol ether derivative | [82] |
| Monogalactosyl diglyceride (112) and digalactosyl diglyceride (DGDG) | Vero cells, HSV-1, HSV-2 PRA ACV HSV-1 IC₅₀ 0.64 μg/mL and HSV-2 IC₅₀ 0.80 μg/mL | HSV-1 IC₅₀ 36.00 μg/mL for 112 and 40.00 μg/mL for DGDG, respectively. HSV-2 IC₅₀ 41.00 μg/mL for 112 and 43.20 μg/mL for DGDG, respectively. | **Clinacanthus nutans** | [83] |
| Methyl (N-benzoyl-(2'R,3'S)-3'-phenylisoserinate) (113) | Vero cells, HSV-1 (MacIntyre strain) CPE ACV IC₅₀ 1 μg/mL, SI > 250 | HSV-1 IC₅₀ 10.7 μg/mL, SI > 46.7 | Taxol derivatives. The activity may be associated with their influence on mitotic division. | [68] |
**Figure 5.** Miscellaneous compounds with antiherpetic activity.

**Table 5.** The overview of polysaccharides with anti-HSV activity.

| Compound | Antitherpetic and Cytotoxicity Assays, Strains, Cells, and Reference Agents | Results | Additional Information | Source |
|----------|--------------------------------------------------------------------------------|--------|------------------------|--------|
| PSP-B2 polysaccharide from *Prunellae Spica* (*Prunella vulgaris* L.) | Vero cells, HSV-1, HSV-2 PRA ACV HSV-1 IC₅₀ 0.78 µM, HSV-2 1.32 µM | HSV-1 IC₅₀ 69 µg/mL HSV-2 IC₅₀ 49 µg/mL | No cytotoxicity even at 1600 µg/mL [84] | |
| *Eucheuma gelatinae* (seaweed) polysaccharide | Vero cells, HSV-1 PRA ACV EC₅₀ HSV-1 (strain F), HSV-2 (strain 333), HSV-1 (strain 106), HSV-1 (strain 153), HSV-1/F, HSV-2/333, HSV-1/106, HSV-1/153, and HSV-1/blue EC₅₀ 0.65, 2.12, 1.11, 1.24, and 1.48 µg/mL, respectively | Effect via activity on early HSV-1 infection. Inhibition of viral DNA synthesis. [85] | |
| Sulfated polysaccharide SP-III from *Sargassum latifolium* | Vero cells, HSV-1 PRA | SP-III 33% and 81% inhibition at 20 μg/mL and 40 μg/mL, respectively. | Glucuronic acid, mannose, glucose, xylose and fucose. |
|---|---|---|---|
| Vero cells, HSV-1 (15577 strain, clinical strain, DM2.1 strain-ACV resistant) PRA | Determination of extracellular virucidal activity Time of addition experiment Virus adsorption assay | Inhibition of replication of both the acyclovir-sensitive and -resistant strains of HSV-1, in a dose-dependent manner, EC₅₀ 1.5–5.3 μg/mL | Fucose, xylose, mannose, glucose, galactose, galactosamine Extracellular virucidal activity only against the ACV-sensitive strains. This compound might inhibit the attachment of the virus to its host cell. |
| ST-F polysaccharide from marine brown algae *Sargassum trichophyllum* | Vero cells, HSV-2 (UW264 strain) PRA | Added to the medium during infection and throughout the incubation (Experiment A) or immediately after viral infection (Experiment B), IC₅₀ 18 and 410 μg/mL, respectively. SI > 280 and >12 for A and B, respectively. | (Fucose and galactose) The main antiviral target of ST-F might be virus adsorption and/or penetration step(s) on the host cell surface. Low cytotoxicity |
| Vero cells, HSV-1 Neutral red dye method ACV EC₅₀ 15.4 ± 5.6 μg/mL | S. *fluitans* EC₅₀ 42.8 ± 4.3 μg/mL and S. *filiformis* EC₅₀ 136.0 ± 12 μg/mL | Without cytotoxicity (1–200 μg/mL) | The activity observed suggests that the degree of sulfation, molecular weight, and carbohydrate nature of these polysaccharides may affect the activity |
| Polysaccharide fractions $C_1^p$ and $C_4^p$ from chlorophyta *Ulva armoricana* | Vero cells, HSV-1 (wild-type strain 17, sensitive to ACV) CPE ACV EC$_{50}$ 0.3 µg/mL | EC$_{50}$ 373.0 ± 20.7 and 320.9 ± 6 µg/mL | Activities correlated to amounts of rhamnose, uronic acids and degree of sulfation. [90] |
| Sp-Am polysaccharide from *Acanthophora muscoides* | Vero cells, HSV-1, HSV-2 CPE | HSV-1 IC$_{50}$ 1.63 µg/mL, SI = 3.5 HSV-2 IC$_{50}$ 3.5 µg/mL, SI = 99.9 | Sulfated polysaccharides from marine seaweeds The possible mechanism of the effect - the inhibition of virus adsorption. [91] |
| SP-Gb polysaccharide from *Gracila riabirdiae* | Vero cells, HSV-1, HSV-2 CPE | HSV-1 IC$_{50}$ 0.75 µg/mL, SI = 1.25 HSV-2 IC$_{50}$ 82.2 µg/mL, SI = 94.40 | Galactose, 3,6-anhydrogalactose, uronic acids, sulfated The adsorption step of HSV-1 to the host cell possible mechanism of action. [92] |
| SP-Sf polysaccharide from *Solieria filiformis* | | HSV-1 IC$_{50}$ 0.6 µg/mL, SI = 1.6 HSV-2 IC$_{50}$ 74.9 µg/mL, SI = 97.5 | Possible inhibiting HSV attachment to cells by direct interaction with viral particles. [93] |
| PSC polysaccharide from marine seaweed *Sphaerococcus coronopifolius* | Vero cells, HSV-1 (wild type strain 17, sensitive to ACV) CPE Time of addition assay Virus adsorption assay ACV SI > 500 | EC$_{50}$ 4.1 µg/mL, SI = 61 | | |
| PBT polysaccharide from marine seaweed *Boergeseniella thuyoides* | | | EC$_{50}$ 17.2 µg/mL, SI = 14.5 | | |
| Sulfated xylogalactofucans and alginic acids from brown algae *Laminaria angustata* | RC-37 cells, HSV-1 (KOS) PRA Time of addition assay Virus adsorption assay Virucidal assay | IC$_{50}$ 0.21–25 µg/mL, SI > 40 > 3225 | | |
| SU1F1 polysaccharide from green algae *Enteromorpha compressa* | HEp-2 cells, HSV-1 (clinical isolate) PRA Time-of-addition assay Inhibition of adsorption assay Inhibition of penetration assay Virucidal assay Acyclovir IC$_{50}$ 2100 µg/mL, SI = 1.21 | IC$_{50}$ 28.25 µg/mL, SI = 35.3 | Chemically altered-sulfated ulvan Broad mechanism of action. [94] |
| Sulfated fucoids (S1-S3) from marine brown alga Padina tetrastomatia | Vero cells, HSV-1 (strains F and B2006), HSV-2 (strain MS)          | HSV-1 and HSV-2 with IC₅₀ in range of 0.30–1.05 μg/mL              | Active during the virus adsorption period                  [95] |
|---------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------------------|
| Virucidal assay                                                | PRA                                                              | B2006 S₃ IC₅₀ 0.6 μg/mL                                         | Degree of sulfation affects the activity                  |
| Effect of treatment period on the antiviral activity           |                                                                 |                                                                 |                                                         |
| Polysaccharides from brown seaweed Stoechospermum marginatum   | Vero cells, HSV-1 (strains F, TK; B2006 and filed strains, syncytial variants arising after selection with a natural carrageenan, syn 13-8 and 14-1), HSV-2 (MS) | HSV-1 (F) EC₅₀ 1.15-50 μg/mL, SI >20 >869                      | Sulfated fucans                                          [96] |
|                                                             | PRA                                                              | HSV-2 (MS) EC₅₀ 0.78 μg/mL, SI >0.57 >50                        | Active during the virus adsorption period.                 |
|                                                             |                                                                 | F₃ B₂₀₀₆, Field, 13-8 and 14-1 strains EC₅₀ 0.95, 1.52, 4.52 μg/mL, SI >1053, >658, >221, >176 | No direct virucidal activity.                            |
|                                                             |                                                                 |                                                                 | No correlation between the antiviral and anticoagulant activity. |
| CiWE CiF₃ polysaccharides from brown seaweed Cystoseira indica  | Vero cells, HSV-1 (strain F), HSV-2 (strain MS)                   | IC₅₀ values in the range of 0.5–2.8 μg/mL                       | Sulfated fucans                                          [97] |
|                                                             | PRA                                                              |                                                                 | Degree of sulfation affects the activity.                 |
|                                                             |                                                                 |                                                                 | No correlation between the antiviral and anticoagulant activity. |
| Sulfated polysaccharide (fucoidan) from brown algae Undaria pinnatifida (Mekabu) | Vero cells, HSV-1 (strain HF), HSV-2 (strain UW-268)              | HSV-1 IC₅₀ and SI A) 2.5 μg/mL, >800; B) 14 μg/mL, >140         | Fucose, galactose                                        [98] |
|                                                             | PRA                                                              | HSV-2 IC₅₀ and SI A) 2.6 μg/mL, >770; B) 5.1 μg/mL, >390        | Other viruses tested.                                    |
|                                                             | A) 1 h after the viral infection, B) immediately after infection |                                                                 |                                                         |
| Polysaccharides (CiWE and F₃) from red seaweed Grateloupia indica | Vero cells, HSV-1 (strain F, TK; B2006 and filed strains, syncytial variants arising after selection with natural carrageenan, syn 13-8 and 14-1), HSV-2 (MS) | HSV-1 (F) IC₅₀ 0.27 μg/mL and HSV-2 (MS), IC₅₀ 0.31 μg/mL      | Sulfated galactans                                       [99] |
|                                                             | PRA                                                              | F₃ B₂₀₀₆, Field, 13-8 and 14-1 strains EC₅₀ 0.89, 0.87, 1.06, 0.81 μg/mL, SI >1123, >1149, >943, >1234 | Degree of sulfation affects the activity.                 |
|                                                             |                                                                 | No direct virucidal activity at 40 μg/mL                        | Possible ability to interfere with the replication cycle. |
| Polysaccharide from *Schizymenia binderi* | Vero cells, HSV-1 (strains F, TK− (B2006), (Field)), HSV-2 (G) PRA | EC₅₀ 0.21-0.76 μg/mL SI > 1000 for all assays No cytotoxicity at 1000 μg/mL | Sulfated galactan Interference with the initial adsorption of viruses to cells, no virucidal activity at 100 μg/mL. [100] |
|------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Polysaccharide from red seaweed *Gigartina skottsbergii* | Vero cells, HSV-1 (strains F, TK− (B2006), (Field), clinical isolates 1213 LCR/94, 374 LCR/94 and 1180 BE/94), HSV-2 (G, clinical isolate 244 BE/94) PRA | 1C: HSV-1 (F) and HSV-2 (G) EC₅₀ 0.7 and 0.5 μg/mL, respectively, SI > 1408 and >2128 1T: HSV-1 (F) and HSV-2 (G) EC₅₀ 0.6 and 0.4 μg/mL, respectively, SI > 1538 and >2439 Clinical isolates EC₅₀ 0.19–2.18 μg/mL | Carrageenans Lack of anticoagulant activity No virucidal activity, effect on virus adsorption [101] |
| Proteoglycan GLPG from *Ganoderma lucidum* (Agaromycetes)—lingzhi mushroom | Vero cells, HSV-1, HSV-2 CPE Virus yield inhibition assay | HSV-1 and HSV-2 EC₅₀ 48 and 56 μg/mL, respectively, SI > 42 and >36 No cytotoxicity at 2000 μg/mL | Proteoglycan GLPG (carbohydrate: protein ratio of 10.4:1) The antiviral activity may be due to its inhibiting HSV attachment to cells, in addition, its inhibition of viral penetration would augment its antiviral activity. [102] |
| Polysaccharide RP from *Portulaca oleracea* | Vero cells, HSV-2 (UW268 strain) PRA A: RP added during infection and throughout the incubation thereafter B: RP added immediately after viral infection. A: EC₅₀ 210 μg/mL, SI = 33 B: EC₅₀ 320 μg/mL, SI = 22 | Virus adsorption and penetration assay | Pectic polysaccharide (RP) Activity against influenza virus tested on MDCK cells. [103] |
| Polysaccharide SPLCf from *Caesalpinia ferrera* | HEP-2 cells, HSV-1 (clinical isolate) PRA | IC₅₀ 405 μg/mL, SI > 7.4. | Sulfated polysaccharide [104] |
### Polysaccharides (ANP, AAP) from *Acanthopanax sciadophylloides*

| Polysaccharides | Vero cells, HSV-2 (UW264 strain) | ANP and AAP IC\(_50\) 52 and 620 μg/mL when added to the medium during infection and throughout the incubation thereafter |
|-----------------|---------------------------------|----------------------------------------------------------------------------------------------------------------|
| PRA             | In vivo anti-HSV-2 effects on female BALB/c mice |
| ACV             | HSV-2 IC\(_50\) 67 and 580 μg/mL when added to the medium immediately after infection. |

*SPLCf showed the effect on several stages of the HSV replication—virus adsorption, the effect on virus particles and the expression of viral protein.*

### Acidic polysaccharide (nostoflan) from terrestrial cyanobacterium *Nostoc flagelliforme*

| Polysaccharide | Vero cells, HSV-1 (HF strain) and HSV-2 (UW268 strain) |
|----------------|---------------------------------------------------------|
| PRA            | A: added during infection and throughout the incubation thereafter |

| Polysaccharide | Vero cells, HSV-1 strain F, TK-B2006 and field strains, HSV-2 strain |
|----------------|----------------------------------------------------------------------|
| PRA            | HSV-1 (strain F, TK-B2006 and field strains) EC\(_50\) 4.2, 2.4, 2.2 μg/mL, SI > 238, >417, >454 |
|                | HSV-2 (strain G) EC\(_50\) 3.0 μg/mL, SI > 333 |

*No cytotoxic effects up to 1000 μg/mL*

*SPLCf showed the effect on several stages of the HSV replication—virus adsorption, the effect on virus particles and the expression of viral protein.*

### Polysaccharide sulfate fraction from *Caulerpa racemosa*

| Polysaccharide | Vero cells, HSV-1 strain F, TK-B2006 and field strains, HSV-2 strain |
|----------------|----------------------------------------------------------------------|
| PRA            | HSV-1 (strain F, TK-B2006 and field strains) EC\(_50\) 4.2, 2.4, 2.2 μg/mL, SI > 238, >417, >454 |
|                | HSV-2 (strain G) EC\(_50\) 3.0 μg/mL, SI > 333 |

*Galactose, glucose, arabinose, and xylose as the major components.*

*Other antiviral activity tested.*

*No anti-thrombin activity.*
Table 6. The overview of peptides with anti-HSV activity.

| Compound                          | Antiviral and Cytotoxicity Assays, Strains, Cells, and Reference Agents | Results                                                      | Additional Information                                                                 | Source |
|-----------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------|----------------------------------------------------------------------------------------|--------|
| **Bacteriocins**                  | Vero cells, HSV-1 (strain EK)                                             | Before adsorption IC₅₀ 235.6 μg/mL, SI 4.6 (for GEn14)       | Bacteria from goat milk Enterococcus durans (GEn09, GEn12, GEn14, and GEn17).          | [108]  |
| (semi-purified)                   | CPE                                                                       | After adsorption IC₅₀ 24.0 μg/mL, SI 17.8 (for GEn17)         |                                                                                        |        |
|                                   | Viral adsorption assay                                                    |                                                              |                                                                                        |        |
| **Subtilosin**                    | Vero cells, HSV-2 (strain G)                                             | At 200 μg/mL a reduction over 99.9% in virus titer          | Bacillus amyloliquefaciens Antiviral and virucidal effect. This compound affects late stages of the viral replicative cycle such as viral glycoprotein intracellular transport. | [109]  |
|                                  | PRA                                                                       | EC₅₀ 18.2 μg/mL, SI 17.4                                    |                                                                                        |        |
|                                  | Virucidal assay                                                           |                                                              |                                                                                        |        |
|                                  | Time-of-addition assay                                                   |                                                              |                                                                                        |        |
|                                  | Indirect immunofluorescence assay                                        |                                                              |                                                                                        |        |
| **Simplicilliumtide J**           | Vero cells, HSV-1                                                         | IC₅₀ 14.0 μM                                                | Deep-Sea-Derived Fungus Simplicillium obclavatum Cyclic peptides                        | [110]  |
| **Verlamelin A**                  | Vero cells, HSV-1                                                         | IC₅₀ 16.7 μM                                                |                                                                                        |        |
|                                  | ACV IC₅₀ 3.0 μM                                                          |                                                              |                                                                                        |        |
| **Verlamelin B**                  |                                                                           | IC₅₀ 15.6 μM                                                |                                                                                        |        |
| **Aspergillipeptide D**           | Vero cells, HSV-1 (strain 15577, ACV resistant clinical isolates HSV-1-106 and HSV-1-153) | HSV-1 (strain 15577) IC₅₀ 9.5 μM                            | Marine gorgonian-derived fungus Aspergillus sp. No cytotoxicity at concentrations tested. Aspergillipeptide D showed activity against acyclovir-resistant HSV-1-106 and HSV-1-153. | [111]  |
|                                  | CPE                                                                       |                                                              |                                                                                        |        |
| **Aspergillipeptide E**           | Vero cells, HSV-1 (strain 15577)                                         | HSV-1 (strain 15577) IC₅₀ 19.8 μM                            |                                                                                        |        |
|                                  | ACV IC₅₀ 3 μM                                                            |                                                              |                                                                                        |        |
| **RC28 (28.25 kDa)**             | BGMK cells, HSV-1 (KOS)                                                  | IC₅₀ 0.078 mg/mL, SI > 32                                   | Edible mushroom Rozites caperata (Cortinarious caperata)                                | [112]  |
| protein                           | CPE                                                                       |                                                              |                                                                                        |        |
| **Pa-MAP**                        | Vero cells, HSV-1                                                         | EC₅₀ 83 μg/mL, SI > 5                                       | Polar fish Pleuronectes americanus                                                    | [113]  |
| Virus titer reduction method | Virucidal assay | ACV at 20 μg/mL PI 99% |
|-----------------------------|----------------|-----------------------|

| Bovine lactoperoxidase      | Vero cells, HSV-1 PRA | At 0.5 mg/mL 100% antiviral effect | Milk hemoprotein |
|-----------------------------|-----------------------|-----------------------------------|------------------|

| Griffithsin                  | Vero cells, HeLa cells, HSV-2 (strain G)  
PrestoBlue cell viability reagent - reading of fluorescence  
Flow cytometry  
Inhibition of adsorption assay  
In vivo HSV-2 murine model | EC₅₀ 5.8 μg/mL (230 nM)  
Griffithsin/carrageenan combination EC₅₀ 3.4 ng/mL | Red alga *Griffithia*  
A lectin with high affinity for mannose-rich N-linked glycans. Griffithsin may block viral entry by binding to HSV-2 glycoprotein D. The griffithsin/carrageenan combination product but not GRFT or CG alone, reduced HSV-2 vaginal infection in mice when given an hour before challenge. |
|-----------------------------|-----------------------|-----------------------------------|------------------|

| Melittin                    | Vero cells, HSV-1  
Virus yield inhibition assay | EC₅₀ 1.35 μM; SI 6.3 | Cationic 26 amino acids peptide isolated from insects—the main component of bee venom |
|-----------------------------|-----------------------|-------------------|--------------------------------|

[114] [115] [116]
4. Natural Products Targeting Enzymes Implicated in HSV Replication

Over the past few decades, structural and mechanistic enzymology played a central role in virology research, where a wide range of enzymes that play a vital role in viral replication, viral transcription or have an impact on the pathogenesis of infection have become imperative drug targets for therapeutic intervention [117,118]. Recently, Čulenová et al. [34] have isolated phenolic compounds from *Morus alba* root bark, kuwanon C (22), kuwanon T (23), kuwanon U (24) and ethyl 2,4-dihydroxybenzoate (37) with clear inhibitory action against HSV-1, with IC50 values ranging from 0.64 to 1.93 μg/mL, while kuwanon E (25) and mulberrofuran B (52) inhibited effectively the replication of HSV-2, with EC50 values of 0.93 and 1.61 μg/mL, respectively. Molecular docking analysis outcomes proved the effects of the active compounds by targeting the HSV-1 DNA polymerase and HSV-2 protease (proposed as competitive inhibitors), which are crucial enzymes that display an important role in the HSV replication cycle.

Geraniol (62), a monoterpenoid active compound detected in *Thymus bovei* Benth. essential oil has shown to possess obvious inhibitory effects on HSV-2 replication (EC50 = 1.92 μg/mL; SI = 109.38) compared with that of standard ACV (EC50 = 1.94 μg/mL; SI = 108.25). This substance, in a molecular docking analysis, has proved to bind to the active site of HSV-2 protease as a competitive inhibitor, and hence uncovered the potential mechanism of action behind the antitherpetic properties against HSV-2 (Figure 6) [57].

![Figure 6](image-url)

**Figure 6.** The two-dimensional (2D) interaction diagram of 62 in the active cavity of HSV-2 protease. Only those amino acid residues implicated in the enzyme stabilization are exposed. Hydrogen bonding and several substantial interactions with amino acid residues are displayed. This figure and its description have been adapted from Hassan et al. [57] with permission, as the article has been published by an MDPI publisher and licensed under an open access Creative Commons CC BY 4.0 license.

In another study, psoromic acid (45), a bioactive, lichen-derived molecule, was tested for its inhibitory action against HSV-1 and HSV-2 [46]. The results advocated that this molecule effectively inhibited HSV-1 (IC50 = 1.9 μM; SI: 163.2) and HSV-2 (EC50 = 2.7 μM; SI: 114.8) replication compared with that of ACV (for HSV-1 IC50 = 2.6 μM; SI: 119.2 and for HSV-2 EC50 = 2.8 μM; SI: 110.7). Also, the inhibition potency of 45 was enhanced through a combination with ACV as a combinatory treatment. Further, the potential mechanism of action against HSV-1 was revealed by in vitro and in silico assays (Figure 7) via inhibiting the HSV-1 DNA polymerase. In an in vitro assay, 45 was proved to be a non-nucleoside inhibitor as well as a competitive inhibitor of the HSV-1 DNA polymerase with respect to dTTP incorporation (IC50 = 0.7 μM; inhibition constant (K) = 0.3 μM) compared with reference drugs aphidicolin (IC50: 0.8 μM; K: 0.4 μM) and ACV triphosphate (ACV-TP) (IC50: 0.9 μM; K: 0.5 μM).
Additionally, molecular docking investigation has revealed the potential mechanism underlying the anti-HSV-2 property of 45 by targeting HSV-2 protease (competitive inhibitor) (Figure 8).

Figure 7. Molecular interaction of psoromic acid (PA, 45) with the active site of HSV-1 DNA polymerase. Amino acid residues involved in HSV-1 DNA polymerase stabilization along with the hydrogen bonding and other essential interactions for enzyme inactivation are presented. The key functional groups of PA that are responsible for anti-HSV-1 DNA polymerase activity are depicted. This figure and its description have been adapted from Hassan et al. [46] with permission, as the article has been published by an MDPI publisher and licensed under an open access Creative Commons CC BY 4.0 license.

Figure 8. Molecular interaction of psoromic acid (PA, 45) with the active site of HSV-2 protease. Amino acid residues involved in HSV-2 protease stabilization along with the hydrogen bonding and other essential interactions for enzyme inactivation are illustrated. Significant functional groups of PA that account for the inhibitory action against HSV-2 protease are presented. This figure and its description have been adapted from Hassan et al. [46] with permission, as the article has been published by an MDPI publisher and licensed under an open access Creative Commons CC BY 4.0 license.
5. General Discussion

In general, from the data analysis, we cannot merely conclude with any broad recommendation for further phytochemical research on specific plant family or genus, just some limited hints connected to specific groups of compounds or plant species. First, we have to mention that there is a relative lack of information concerning in vivo testing of compounds assayed in the Vero cell model system against HSV, as described, for example, here [119]. The methodology for testing in vitro anti-HSV activity is commonly based on the assays using the Vero cell line (kidney epithelial cells extracted from an African green monkey (Chlorocebus sp.). Vero cells are widely acknowledged to be well-suited for testing antiviral activity, as these cells do not secrete interferon α or β as a response to viral infection, while possessing the INF-α/β receptors, and therefore behave normally after the addition of exogenous interferon [120]. The overall stability and susceptibility of Vero cells to many pathogens, including HSV, makes these cells a very useful tool for testing new potential anti-HSV compounds.

The methodology for testing the anti-HSV activity used in the covered literature search is relatively uniform, allowing the detection of potential hits and finding candidates for antiviral research [121]. The main methods used are analyses of the viability of infected and non-infected cells, the plaque reduction assay, virus cytopathic effect monitoring [122], real-time PCR, quantification of intracellular viral DNA load [123] and the following calculation of selectivity indices. Virus multiplication can also be monitored by ELISA analysis of antigen expression in cell culture. Modifications of these methods, using the sophisticated timing of anti-HSV drug candidate application and further analysis, can give additional information about HSV attachment and penetration to cells [124,125].

The very common therapeutic standard used as the positive control of anti-HSV assays is acyclovir [126]. As it is evident from our literature search and other materials, both HSV-1 and HSV-2, including clinical strains, are sensitive to acyclovir when propagated in Vero cells, with IC₅₀ values at low-micromolar concentrations (or micrograms per mL) and selectivity indices reaching values up to 1000 or greater.

According to our literature research, there is an interest in finding new or alternative anti-HSV compounds, represented, for example, by the above-mentioned review published in 2015 [25]. We organized an additional search for anti-HSV natural compounds and gathered information about approximately 100 low-molecular secondary metabolites, obtained from both plants and marine organisms, and also high-molecular polymers represented by a number of sulfated polysaccharides, mainly from marine organisms (algal compounds) and peptides of mainly microbial origin.

Within the compounds mentioned, the most frequent groups with anti-HSV properties are groups of phenolic compounds, comprising a set of simple phenols, flavonoids (mainly dietary flavonoids) and tannins (Table 1). Based on the results of the concurrent analysis and a comparison with previously summarized reports about anti-HSV-activity [22–25], we can conclude that tannins possess activity comparable to standard acyclovir. Compounds 40 and 41 show activity almost 2000 greater and can possibly prevent the attachment of viral particles to the cells and stop the virus’ penetration into the cell [43]. Similarly, some flavonoid aglycones displayed promising results, showing greater effects than acyclovir and greater selectivity. Moreover, according to our recent findings, we can deduce that flavanols are showing greater activity than flavones. This beneficial effect could be possibly subscribed to the 3-OH hydroxy substitution [27]. Furthermore, the treatment of cells with epicatechin gallate (8) and galangin (11) before HSV adsorption led to some increase in inhibition as determined, indicating that an intracellular activity against the virus may also be involved.

The dual antiviral and antibacterial activity can be beneficial, for example, in the treatment of oral or labial herpetic lesions, which can be relatively easily complicated by secondary bacterial infections. From Table 1, we can deduce that one of the most active phenolic compounds against HSV-1 was kuwanon T (23), with IC₅₀ 0.64 μg/mL (corresponding to 1.5 μM) and SI 328.1. Kuwanon T (23) has also shown promising antibacterial activity against several Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA) and Enterococcus faecalis. The MIC values of
compound 23 ranged from 4–8 μg/mL which exceeded the activity of standard antibiotics ampicillin and ciprofloxacin [34]. Another promising phenolic compound against HSV-1 is galangin (11), with IC₅₀ 2.5 μM and SI 400. Further, galangin (11) has shown bacteriostatic activity against S. aureus (ATCC 25923) with MIC value 32 μg/mL [127]. Another phenolic with equal antiviral activity—naringin (17)—showed no inhibitory effect on several Gram-positive and Gram-negative, even at a concentration of 250 μM [128]. This dual ability or disability can therefore be a secondary criterion for the potential use of natural anti-herpetic compounds and further research on their activity.

The terpenoids form a relatively wide group of compounds, represented by a number of different skeletons. Each group of terpenoids—monoterpenes, sesquiterpenes, diterpenes and triterpenes (including steroidal compounds)—gave us at least one positive hint in the search. The least abundant are monoterpenes, that are represented only by cypellocarpin C (63) isolated from E. globulus (63, arising from the combination of monocyclic monoterpenes with a methylchromone), geraniol (64), here obtained from T. bowei essential oil [57], showing effects against HSV-2, both IC₅₀ and SI, greater than acyclovir [58], and (+)-rhodonoid C (64) (a cross-metabolite of monoterpene and polyketide [59]). In comparison to acyclovir, positive results were also obtained for cucurbitacin B (94) [73], meroditerpenes from Brazilian seaweed Stypopodium zonale 79 and 80 [67], dollabene diterpenes (85 and 86) from brown alga Dicyota piffii [70], which were inhibiting reverse transcriptase of HSV-1, as well as for halistanol derivatives (95–97), a steroidal type compound obtained from Brazilian marine sponge Petromica citrina [74] which, interestingly, showed a synergistic effect when tested together with acyclovir, but a lower selectivity index when tested alone. Triterpenic tereticonate (106) from E. globulus, showed an interesting effect against HSV-1 with an SI slightly better than acyclovir [58].

Cucurbitacin B (94) is one of the most potent antiviral triterpenoids (IC₅₀ = 0.94 μM and SI = 127.7), as shown in Table 3. This compound is also a very effective antibacterial agent—its MIC values against S. aureus and MRSA were found to be 0.20 and 0.12 μg/mL, respectively [73]. However, cypellocarpin C (63), an effective terpenoid molecule against HSV-2 with IC₅₀ = 0.73 μg/mL and SI > 287.7, did not show any antibacterial activity against several Gram-positive and Gram-negative bacteria [58], and, as in the case of phenolics, this can be a selective criterion for further research.

Polysaccharides, heterogeneous natural compounds with promising anti-HSV activity, were reviewed in 2009 [129]. Many of them were isolated from marine seaweeds, especially Chromophyta (brown algae) and Rhodophyta (red algae), and their anti-HSV activities were evaluated and confirmed recently (as visible in Table 5). From the structural point of view, most of them are sulfated polysaccharides with a different degree of sulphation. The degree of sulphation was found to be important for the anti-HSV effect, however, a question remains around the anticoagulant activity of such compounds. Several studies found no correlation between anticoagulant and antiviral activity of sulphated polysaccharides, and such activity would be clinically important only after absorption of the compound into an organism, not during local application. The benefit of anti-HSV polysaccharides can be observed (when measured and calculated) in their high selectivity index. The examples of promising compounds can be partially cyclized μ/ν-carrageenan from red seaweed Gigartina skottsbergii [101], sulfated galactans from Schizymenia binderi [100], and nostoflan, the acidic polysaccharide from terrestrial cyanobacterium Nostoc flagelliforme [106].

The last separated reviewed group of compounds are peptides, obtained from various sources, including bacteria, deep-sea fungi, or edible mushrooms. Griffithsin, isolated from red alga Griffithisia (family Wrangeliaceae), appears to be very effective against HSV-2, with effects at submicromolar concentrations. Furthermore, griffithsin can be possibly combined with carrageenan and effectively used topically in vivo [115]. Among the potent antiviral peptides against HSV-1 is also melittin, with IC₅₀ 1.35 μM and SI 6.3. This peptide acts also as antibacterial—when MRSA was treated with melittin at a concentration of 25 μg/mL, the total number of bacteria decreased by ~2.5–3 log CFU [130].

From the reviewed articles, all potential mechanisms by which natural products-derived chemicals induced anti-HSV properties have been documented and highlighted, as shown in Figure 9. In the reviewed articles, the majority of assays were basically performed to evaluate the
concentration of test compounds necessary to reduce the number of plaques formed in cells and to calculate the selectivity index from the corresponding cytotoxic effect of the test compound on Vero cells. For some compounds, authors performed additional assays to gain deeper insight into the mechanism of action. As an example, chebulagic acid (40) and chebulinic acid (41) were observed to prevent the attachment and penetration of HSV-2 into Vero cells [43]. Curcumin (56) was detected to inhibit HSV adsorption and replication [51], while houttuynoid A (21) was noted to block viral membrane fusion [32]. Another good example is the research on prenylated phenol kuwanon X (51) [50]. Compound 51 did not inactivate cell-free HSV-1 but inhibited the cellular adsorption and penetration of HSV-1 viral particles. Following viral penetration, 51 reduced the expression of HSV-1 IE and L genes and decreased the synthesis of HSV-1 DNA. Furthermore, 51 inhibited the HSV-1-induced nuclear factor (NF)-κB activation through blocking the nuclear translocation and DNA binding of NF-κB. The study of Lee et al. [28] gave some insight into the effect of flavonoids, showing the ability of quercetin (19), a “prototype” of flavonoid, to inhibit the expressions of HSV proteins (gD, ICP0) and genes (ICP0, UL13, UL52), and specifically suppress the expression of TLR-3 and inhibit the transcription factors NF-κB and IRF3 [28]. The antiviral activity of halistanol derivatives (96 and 97) against HSV-1 is enabled by the inhibition of viral particles’ attachment and penetration; the virucidal effect was also observed. Further analysis showed changes in the levels of proteins ICP27 and the gD of HSV-1. These compounds also act synergistically or with acyclovir [74].

Figure 9. An infographic illustrates the potential mechanisms by which bioactive natural products induce antiviral properties against HSV infection.

6. Take-Home Messages

Based on the collected data obtained from the reviewed articles, we may summarize the most promising bioactive natural products that could be used as templates for the further development of anti-HSV drugs through the preparation of analogs using chemical modification processes such as total or semi-synthesis along with combinatorial synthesis, especially with nanoparticles (Table 7). It should be emphasized that we selected bioactive natural products based on the mechanisms of action or types of inhibition induced (against the replication of HSV and its associated steps, or the enzymes involved in the HSV replication cycle). Additionally, these compounds were also selected based on their structure–activity relationship (SAR) that indicates functional groups, which are accountable for the enhanced anti-HSV activity. Based on the above-mentioned selection criteria, where the mechanisms of action, types of inhibition, and SAR are highlighted, we might aid medicinal chemists
in the design and synthesis of novel and potent compounds useful for the development of anti-HSV drugs.

Table 7. Bioactive natural products reported as inducing potent anti-HSV properties.

| Chemical Class | Compound | Mechanisms of Action or Types of Inhibition | Structure–Activity Relationship (SAR) |
|----------------|----------|---------------------------------------------|--------------------------------------|
| Flavan-3-ol (flavonoid) | Epicatechin gallate (ECG) (6) | Inhibition of viral adsorption. | — |
| Flavonol (flavonoid) | Galangin (11) | Inhibition of viral adsorption. | — |
| Flavonol (flavonoid) | Quercetin (19) | Inhibition of the expressions of HSV proteins (gD, ICP0) and genes (ICP0, UL13, UL52). Additionally, this molecule suppressed the expression of TLR-3 and inhibited the transcriptional factors NF-κB and IRF3. | — |
| Flavonoid Phenolics | Houttuynoid A (21) | Blocking viral membrane fusion. | — |
| | kuwanon C (22), kuwanon T (23), kuwanon U (24), | Inhibition of HSV-1 and HSV-2 replication (in vitro) and inactivation of HSV-1 DNA polymerase and HSV-2 protease (proposed as competitive inhibitors via in silico assay). | Hydroxyl, carbonyl, and methyl groups along with phenyl ring (proposed as functional groups via in silico assays). |
| | kuwanon E (25), and ethyl 2,4-dihydroxybenzoate (37) | | |
| Alkyl derivatives of gallic acid | Octyl gallate (39) | Inhibition of multiplication of HSV-1 and suppression of formation of virus progeny at early stages (within 6 h post-infection) in the infected cells. | Alkyl moieties. |
| Tannins | Chebulagic acid (40) and chebulinic acid (41) | Avoiding the attachment and penetration of HSV-2 into Vero cells. | — |
| | | Inhibition of HSV-1 and HSV-2 replication and inactivation of HSV-1 DNA polymerase (competitive inhibitor via in vitro and in silico experiments). Also, via in silico assay, inactivates HSV-2 protease (competitive inhibitor). | Hydroxyl, carbonyl, and methyl groups along with phenyl ring (proposed as functional groups via in silico assays). |
| β-ocinol depsidone, a type of phenolic compound | Psoromic acid (45) | Anti-HSV activity through multiple modes of action (impeded cellular adsorption and penetration of HSV-1 viral particles). After viral penetration, this agent decreased the expression of HSV-1 IE and L genes and diminished the synthesis of HSV-1 DNA. Moreover, this molecule prevented the HSV-1-induced nuclear factor (NF)-κB activation via obstructing the nuclear translocation and DNA binding of NF-κB. | — |
| Stilbene derivative | Kuwanon X (51) | Inhibition of adsorption and replication of HSV. | Hydroxyl groups (assessed as functional groups). |
| Flavonoid | Curcumin (56) | Inhibition of viral protein expression. | Hydroxyl and methyl groups (proposed as functional groups via in silico assay). |
| Alkaloid | Harmine (59) | Inhibition of HSV-2 replication (in vitro assay) and inactivation of HSV-2 protease (in silico assay). | Sulfate groups (assessed as functional groups). |
| Monoterpenoid | Geraniol (62) | Suppression of HSV-1 attachment and penetration into the host cells. These substances also impair the levels of ICP27 and gD proteins of HSV-1. | Carboxyl and hydroxyl groups along with sugar moiety (assessed as functional groups). |
| Steroids | Halistanol sulfate (96) and halistanol sulfate C (97) | The compound was detected to be an effective inducer of the autophagy activator Beclin 1, which creates a resistance to HSV-1 replication. | Methoxy and carboxy groups at C-20 were |
| Triterpene glycoside | Glycyrrhizic acid (98) | Inhibition of HSV-1 replication. | |
| Triterpenoid | Methylester of glycyrrhetic acid (102) | | |
| Pentacyclic triterpenoid          | Oleanolic acid (103)                  | Inhibition of HSV-1 and HSV-2 multiplication at the early stage. |
|---------------------------------|--------------------------------------|------------------------------------------------------------------|
| Spiroketal-enol ether derivative | (E)-2-(2,4-hexadiynylidene)-1,6-dioxaspiro[4,5] dec-3-ene (111) | Suppression of viral gene expression and reduction of viral protein accumulation within infected cells. |
| Taxol derivatives               | Methyl (N-benzoyl-(2’R,3’S)-3’-phenylisoserinate) (113) and N-benzyol-(2’R,3’S)-3’-phenylisoserine (114) | Inhibition of HSV-1 replication (the inhibitory activity might be related to the impact on the mitotic division). |
| Polysaccharides                 | Polysaccharides and sulfated polysaccharides | Multiple mechanisms of action (inhibition of HSV replication, inhibition of virus adsorption, suppression of gene expression, suppression of HSV attachment and penetration into the host cell). This antiviral agent alters the late stages of the viral replicative cycle such as viral glycoprotein intracellular transport. |
| Cyclic peptide                 | Subtilosin                           | Sugar moieties and sulfate groups.                                |
| Peptide                        | Griffithsin                          | Blocking viral entry by attaching with HSV-2 glycoprotein D.     |

This table digests the most promising bioactive natural products that have been shown to possess potent anti-HSV activity based on their mechanisms of action, types of inhibition, and SAR, which have been displayed in this review. SAR: Structure–activity relationship that signifies functional groups which are responsible for the improved anti-HSV activity. (—): Data not provided in the articles that have been cited in this review.

7. Concluding Remarks and Future Insights

Currently, there are no effective licensed vaccines available for the treatment of herpesviruses infections, and financial support for their development is running short. Studies on novel anti-HSV activities remain a crucial area in drug discovery, since the currently used medications have failed to induce an effective treatment due to the establishment of drug resistance, and there are still a lot of challenges to developing new antiviral drug candidates. Therefore, there is an urgent demand to search for new sources that provide less resistance and reduce unwanted effects. Natural products, as a vast source of biologically active molecules, have proven to induce promising inhibitory activities against HSV infection, and hence, in this paper, we highlighted and summarized exclusively the recent investigations on the most promising compounds derived from various natural origins that can be used as promising and effective antivirals for the treatment of diseases caused by HSV; these are in vitro and in vivo studies based on several assay systems. Additionally, the data depicted in this paper demonstrate a notable impact of structural variations, as well as the analysis of proposed structure–activity relationships, and disclosed that the inhibitory activity profile of natural-derived molecules relies upon the position and nature of their substituents. Despite relatively few isolated antiviral agents from natural sources advancing to become clinically successful drugs, these unique compounds could be applied as models for the preparation of analogs using chemical modification procedures such as total or combinatorial synthesis, or the alteration of biosynthetic pathways. More research in this field is greatly needed to achieve the design and optimization of potent and selective antiviral drugs with promising levels of activity, reduced adverse effects, low toxicity, and enhanced stability. It is known that clinically used antiviral drugs do not heal the disease while modifying the clinical course of the infection by suppressing viral replication and subsequent epithelial damage. Thus, there is an imperative need for comprehensive management of HSV infections based on the obstruction of transmission, suppression of recurrence, viral shedding and complications, and modification of clinical, and promotion of treatment, courses. Moreover, the
use of natural products with an accepted level of activity against HSV in combination with synthetic nucleoside analogs (as a combinatorial treatment) is another valuable option for the therapy of HSV infection; however, these studies are still limited or have yet to be validated. Therefore, all levels of research, including basic-, clinical-, and population-levels, require continued financial support to promote the development and implementation of effective natural anti-HSV drugs with proper pharmacokinetics, pharmacodynamics, hydrolytic stability, and free toxicological profiles (all these assessments should be taken into consideration with all administered forms of the evaluated drug).

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References

1. Parker, F.; Nye, R.N. Studies on Filterable Viruses: II. Cultivation of Herpes Virus. Am J Pathol. 1925, 1, 337–340.
2. Nahmias, A.J.; Dowdle, W.R. Antigenic and biologic differences in herpesvirus hominis. Prog. Med. Virol. 1968, 10, 110–159.
3. Sanders, J.E.; Garcia, S.E. Pediatric herpes simplex virus infections: An evidence-based approach to treatment. Pediatr. Emerg. Med. Pract. 2014, 11, 1–19.
4. Miller, A.S.; Bennett, J.S. Challenges in the care of young infants with suspected neonatal herpes simplex virus. Hosp. Pediatr. 2015, 5, 106–108.
5. Widener, R.W.; Whitley, R.J. Herpes simplex virus. Handb. Clin. Neurol. 2014, 123, 251–263.
6. Akinyi, B.; Odhiambo, C.; Otieno, F.; Inzaule, S.; Oswago, S.; Kerubo, E.; Ndigo, R.; Zeh, C. Prevalence, incidence and correlates of HSV-2 infection in an HIV incidence adolescent and adult cohort study in western Kenya. PLoS ONE. 2017, 12, e017890.
7. Memish, Z.A.; Almasri, M.; Chentoufi, A.A.; Al-Tawfiq, J.A.; Al-Shangiti, A.M.; Al-Kabbani, K.M.; Otaibi, B.; Assirri, A.; Yezli, S. Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 and Coinfection with HIV and Syphilis: The First National Seroprevalence Survey in Saudi Arabia. Sex. Trans. Dis. 2015, 42, 526–532.
8. Birkmann, A.; Zimmermann H. HSV antivirals - current and future treatment options. Curr. Opin. Virol. 2016, 18, 9–13.
9. Kenny, K.; Leung, W.; Stephanson, K.; Ross, S. Clinical practice in prevention of neonatal HSV infection: a survey of obstetrical care providers in Alberta. J. Obstet. Gynaecol. Can. 2013, 35, 131–137.
10. Johnston, C.; Koele, D.M.; Wald, A. Current status and prospects for development of an HSV vaccine. Vaccine. 2014, 32, 1553–1560.
11. Zhu, X.P.; Muhammad, Z.S.; Wang, J.G.; Lin, W.; Guo, S.K.; Zhang, W. HSV-2 vaccine: current status and insight into factors for developing an efficient vaccine. Viruses. 2014, 6, 371–390.
12. Hassan, S.T.S.; Šudomová, M.; Masarčíková, R. Herpes simplex virus infection: an overview of the problem, pharmacologic therapy and dietary measures. Ceska Slov. Farm. 2017, 66, 95–102.
13. Knipe, D.M.; Cliffe, A. Chromatin control of herpes simplex virus lytic and latent infection. Nat. Rev. Microbiol. 2008, 6, 211–221.
14. Roizman, B.; Whitley, R.J. An inquiry into the molecular basis of HSV latency and reactivation. Annu. Rev. Microbiol. 2013, 67, 355–374.
15. Cliffe, A.R.; Garber, D.A.; Knipe, D.M. Transcription of the herpes simplex virus latency-associated transcript promotes the formation of facultative heterochromatin on lytic promoters. J. Virol. 2009, 83, 8182–8190.
16. Cliffe, A.R.; Arbuckle, J.H.; Vogel, J.L.; Geden, M.J.; Rothbart, SB.; Cusack, C.L.; Strahl, B.D.; Christie, T.M.; Deshmukh, M. Neuronal Stress Pathway Mediating a Histone Methyl/Phospho Switch is Required for Herpes Simplex Virus Reactivation. Cell Host Microbe. 2015, 18, 649–658.
17. Johnston, C.; Corey, L. Current Concepts for Genital Herpes Simplex Virus Infection: Diagnostics and Pathogenesis of Genital Tract Shedding. Clin. Microbiol. Rev. 2016, 29, 149–161.
18. Xingli, X.u.; Ying, Zhang.; Qihan, L.i. Characteristics of herpes simplex virus infection and pathogenesis suggest a strategy for vaccine development. Rev. Med. Virol. 2019, 29, 2054.

19. Michael P Nicoll, João T Proença, Stacey Efstatiou. The molecular basis of herpes simplex virus latency. FEMS Microbiol. Rev. 2012, 36, 684–705.

20. Mancini, M.; Vidal, S.M. Insights into the pathogenesis of herpes simplex encephalitis from mouse models. Mamm. Genome. 2018, 29, 425–445.

21. Egan, K.P.; Wu, S.; Wigdahl, B.; Jennings, S.R. Immunological control of herpes simplex virus infections. J. Neurovirol. 2013, 19, 328–345.

22. Vlietinck, A.J.; De Bruyne, T.; Vanden Berghe, D. A. Plant substances as antiviral agents. Curr. Org. Chem. 1997, 1, 307–344.

23. Cheng, C.-L.; Xu, H.-X. Antiviral agents from traditional Chinese medicine against herpes simplex virus. J. Trad. Med. 2005, 22, 133–137.

24. Chattopadhyay, D. Ethnomedicinal antivirals: scope and opportunity. Edited by Ahmad, I.; Aqil, F.; Owais, M. Modern Phytomedicine. 2006, 313-339.

25. Hassan, S.T.; Masarčíková, R.; Berchová, K. Bioactive natural products with anti-herpes simplex virus properties. J. Pharm. Pharmacol. 2015, 67, 1325–1336.

26. Savi, I.A.; Barardi, C.R.; Simões, C.M. Evaluation of antitherpetic activity and genotoxic effects of tea catechin derivatives. J. Agric. Food Chem. 2006, 54, 2552–2557.

27. Lu, S.Y.; Rhim, J.Y.; Park, W.B. Antitherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro. Arch. Pharm. Res. 2005, 28, 1293–1301.

28. Lee, S.; Lee, H.H.; Shin, Y.S.; Kang, H.; Cho, H. The anti-HSV-1 effect of quercetin is dependent on the suppression of TLR-3 in Raw 264.7 cells. Arch. Pharm. Res. 2017, 40, 623–630.

29. Medini, F.; Megdiche, W.; Mshvidladze, V.; Pichette, A.; Legault, J.; St-Gelais, A.; Ksouri R. Antiviral-guided fractionation and isolation of phenolic compounds from Limonium densiflorum hydroalcoholic extract. C. R. Chim. 2016, 19, 726–732; DOI: 10.1016/j.crci.2016.03.006.

30. Pradhan, P.; Nyugen, M.L. Herpes simplex virus virucidal activity of MST-312 and epigallocatechin gallate. Virus Res. 2018, 2, 93–98.

31. Li, J.J.; Chen, G.D.; Fan, H.X.; Hu, D.; Zhou, Z.Q.; Lan, K.H.; Zhang, H.P.; Maeda, H.; Yao, X.S.; Gao, H. Houttuynoid M, an Anti-HSV Active Houttuynoid from Houttuynia cordata Featuring a Bis-houttuynin Chain Tethered to a Flavonoid Core. J. Nat. Prod. 2017, 80, 3010–3013.

32. Li, T.; Liu, L.; Wu, H.; Chen, S.; Zhu, Q.; Gao, H.; Yu, X.; Wang, Y.; Su, W.; Yao, X.; Peng, T. Anti-herpes simplex virus type 1 activity of Houttuynoid A, a flavonoid from Houttuynia cordata Thunb. Antiviral. Res. 2017, 144, 273–280.

33. Argenta, D.F.; Silva, I.T.; Bassani, V.L.; Koester, L.S.; Teixeira, H.F.; Simões, C.M. Antitherpetic evaluation of soybean isoflavonoids. Arch. Virol. 2015, 160, 2335–2342.

34. Čulenová, M.; Syčrová, A.; Hassan, S.T.S.; Berchová-Bímová, K.; Svobodová, P.; Helclová, A.; Michnová, H.; Hošek, J.; Vasil’ev, H.; Suchý, P.; Kuzminová, G.; Svajdlenka, E.; Gajdžíkov, J.; Čížek, A.; Suchý, V.; Šnejkál, K. Multiple In vitro biological effects of phenolic compounds from Morus alba root bark. J. Ethnopharmacol. 2019, 248, 112296.

35. Fritz, D.; Venturi, C. R.; Carginn, S.; Schripsema, J.; Roehe, P.M.; Montanha, J.A.; von Poser, G. L. Herpes virus inhibitory substances from Hypericum connotatum Lam., a plant used in southern Brazil to treat oral lesions. J. Ethnopharmacol. 2007, 113, 517–520.

36. Ojha, D.; Das, R.; Sobia, P.; Dwivedi, V.; Ghosh, S.; Samanta, A.; Chattopadhyay, D. Pedilanthus lithyaloides Inhibits HSV Infection by Modulating NF-kB Signaling. PLoS One. 2015, 10, e0139338.

37. de Oliveira, A.; Prince, D.; Lo, C.Y.; Lee, L.H.; Chu, T.C. Antiviral activity of theflavin digallate against herpes simplex virus type 1. Antiviral. Res. 2015, 118, 56–67.

38. Likhitwitayawuid, K.; Chaiwiriya, S.; Srirularak, B.; Lipipun, V. Antitherpetic flavones from the heartwood of Artocarpus gomezianus. Chem. Biodivers. 2006, 3, 1138–1143.

39. El-Toumy, S.A.; Saliba, J. Y.; El-Kashak, W.A.; Marty C.; Bedoux, G.; Bourgougnon, N. Antiviral effect of polyphenol rich plant extracts on herpes simplex virus type 1. Food Sci. Human Wellness. 2018, 7, 91–101.

40. Li, Y.; Leung, K.T.; Yao, F.; Ooi, L.S.M.; Ooi, V.E.C. Antiviral flavans from the leaves of Pithecellobium dyspearia. J. Nat. Prod. 2006, 69, 833–835.
41. Boff, L.; Silva, I.T.; Argenta, D.F.; Farias, L.M.; Alvarenga, L.F.; Pádua, R.M.; Braga, F.C.; Leite, J.P.; Kratz, J.M.; Simões, C.M. Strynchos pseudoquina A. St. Hil.: a Brazilian medicinal plant with promising in vitro antithrusp activity. J. Appl. Microbiol. 2016, 121, 1519–1529.

42. Uozaki, M.; Yamasaki, H.; Katsuyama, Y.; Higuchi, M.; Higuti, T.; Koyama, A.H. Antiviral effect of octyl gallocate against DNA and RNA viruses. Antiviral Res. 2007, 73, 85–91.

43. Kesharwani, A.; Polachira, S.K.; Nair, R.; Mishra, N.N.; Gupta, S.K. Anti-HSV-2 activity of Terminalia chebula Retz extract and its constituents, chebulagic and chebulinic acids. BMC Complement Altern. Med. 2017, 17, 110.

44. Lavoie, S.; Côte, I.; Pichette, A.; Gauthier, C.; Quellet, M.; Nagau-Lavoie F.; Mshvidadze, V.; Legault, J. Chemical composition and anti-herpes simplex virus type 1 (HSV-1) activity of extracts from Cornus canadensis. BMC Complement. Altern. Med. 2017, 17, 123.

45. Hassan, S.T.S.; Švajdlenka, E.; Berchová-Bimová, K. Hibiscus sabdariffa L. and Its Bioactive Constituents Exhibit Antiviral Activity against HSV-2 and Anti-enzymatic Properties against Urease by an ESI-MS Based Assay. Molecules. 2017, 22, 722.

46. Hassan, S.T.S.; Šudomová, M.; Berchová-Bimová, K.; Šmejkal, K.; Echeverría, J. Psoromic Acid, a Lichen-Derived Molecule, Inhibits the Replication of HSV-1 and HSV-2, and Inactivates HSV-1 DNA Polymerase: Shedding Light on Antitherapeutic Properties. Molecules. 2019, 24, 2912.

47. Thongchua, B.; Tragoolpua, Y.; Sangthong, P.; Trisuwan, K. Antiviral carboxylic acids and naphthoquinones from the stems of Rhinacanthus nasutus. Tetrahedron Lett. 2015, 56, 5161–5163.

48. He, Y.C.; Lu, Z.H.; Shi, P.; Hao, J.C.; Zhao, Z.J.; Xie, H.T.; Mao, P.; Chen, S.J. Anti-herpes simplex virus activities of bioactive extracts from Antrodia camphorata mycelia. Antivir. Ther. 2016, 21, 377–383.

49. Huang, Z.; Nong, X.; Ren, Z.; Wang, J.; Zhang, X.; Qi, S. Anti-HSV-1, antioxidant and anti-tumour phenolic compounds from the deep-sea-derived fungus Aspergillus versicolor SCSIO 41502. Bioorg. Med. Chem. Lett. 2017, 27, 787–791.

50. Ma, F.; Shen, W.; Zhang, X.; Li, M.; Wang, Y.; Zou, Y.; Li, Y.; Wang, H. Anti-HSV Activity of Kuwanon X from Mulberry Leaves with Genes Expression Inhibitory and HSV-1 Induced NF-κB Deactivated Properties. Biol. Pharm. Bull. 2016, 39, 1667–1674.

51. Cavalcanti, J.F.; de Araujo, M.F.; Gonçalves, P.B.; Romeiro, N.C.; Villela Romanos, M.T.; Curcino Vieira, I.J.; Braz-Filho, R.; de Carvalho, M.G.; Sanches, M.N.G. Proposed anti-HSV compounds isolated from Simiria species. Nat. Prod. Res. 2017, 1–4.

52. Flores, D.J.; Lee, L.H.; Adams, S.D. Inhibition of Curcumin-Treated Herpes Simplex Virus 1 and 2 in Vero Cells. Adv. Microbiol. 2016, 06, 276–287.

53. Rajtar, B.; Skalicka-Wozniak, K.; Švájetek, L.; Stec, A.; Boguszewska, A.; Polz-Dacewicz, M. Antiviral effect of compounds derived from Angelica archangelica L. on Herpes simplex virus-1 and Coxsackievirus B3 infections. Food Chem. Toxicol. 2017, 109, 1026–1031.

54. Beneziri, R.; Bouslama, L.; Lapetti, A.; Hammami, M.; Smaoui, A.; Limam, F. Anti HSV-2 activity of Peganum harmala (L.) and isolation of the active compound. Microb. Pathog. 2018, 114, 291–298.

55. Hutterer, C.; Milbradt, J.; Hamilton, S.; Zaja, M.; Leban, J.; Henry, C.; Vitt, D.; Steingruber, M.; Sonntag, E.; Zeitsträger, I.; Bahsi, H.; Stamminger, T.; Rawlinson, W.; Strobl, S.; Marschall, M. Inhibitors of dual-specificity tyrosine phosphorylation-regulated kinases (DYRK) exert a strong anti-herpes viral activity. Antiviral. Res. 2017, 143, 113–121.

56. Zalilawati, M. R.; Andriani, Y.; Shaari, K.; Bourougnon, N.; Ali, A.M.; Muhammad, T.S.T.; Mohamad, H. Induction of apoptosis and anti HSV-1 activity of 3-(Phenethylamino) demethyl(ox)jaaptamine from a Malaysian Aaplos aapts. J. Chem. Pharm. Res. 2015, 7, 330–341.

57. Hassan, S.T.S.; Berchová-Bimová, K.; Šudomová, M.; Malanik, M.; Šmejkal, K.; Rengasamy, K.R.R. In Vitro Study of Multi-Therapeutic Properties of Thymus bovei Benth. Essential Oil and Its Main Component for Promoting Their Use in Clinical Practice. J. Clin. Med. 2018, 7, 283.

58. Brezání, V.; Leláková, V.; Hassan, S.T.S.; Berchová-Bimová, K.; Nový, P.; Klouček, P.; Maršík, P.; Dall’Acqua, S.; Hošek, J.; Šmejkal, K. Anti-Infectivity against Herpes Simplex Virus and Selected Microbes and Anti-Inflammatory Activities of Compounds Isolated from Eucalyptus globulus Labill. Viruses. 2018, 10, 360.

59. Liao, H.B.; Huang, G.H.; Yu, M.H.; Lei, C.; Hou, A.J. Five Pairs of Meroterpenoid Enantiomers from Rhododendron capitatum. J. Org. Chem. 2017, 82, 1632–1637.
60. Cagno, V.; Sgorbini, B.; Sanna, C.; Caglierio, C.; Ballero, M.; Civra, A.; Donalisio, M.; Bicchi, C.; Lembo, D.; Rubiollo, P. In vitro anti-herpes simplex virus-2 activity of Salvia desoleana Atzei & V. Picci essential oil. PLoS One. 2017, 12, e0172322.

61. Ghannadi, A.; Fattahian, K.; Shokoohinia, Y.; Behbahani M.; Shahnoosh, A. Anti-Viral Evaluation of Sesquiterpene Coumarins from Ferula assa-foetida against HSV-1. Iran. J. Pharm. Res. 2014, 13, 523–530.

62. Krawczyk, E.; Łuczak, M.; Kobus, M.; Bańka, D.; Daniewski, W. Antiviral Activity of N-Benzylophenylisoserinates of Lactarius Sesquiterpenoid Alcohols in vitro. Planta. Med. 2003, 69, 552–554.

63. Rezeng, C.; Yuan, D.; Long, J.; Suonan, D.; Yang, F.; Li, W.; Tong, L.; Jiumei, P. Alantolactone exhibited anti-herpes simplex virus 1 (HSV-1) action in vitro. Biosci. Trends. 2015, 9, 420–422.

64. Tsai, Y.C.; Cheng, Y.B.; Lo, I.W.; Cheng, H.H.; Lin, C.J.; Hwang, T.L.; Kuo, Y.C.; Liou, S.S.; Huang, Y.Z.; Kuo, Y.H.; Shen, Y.C. Seven new sesquiterpenoids from the fruits of Schisandra sphenanthera. Chem. Biodivers. 2014, 11, 1053–1068.

65. Rédei, D.; Küsz, N.; Rafai, T.; Bogdanov, A.; Burián, K.; Csorba, A.; Mándi, A.; Kurtán, T.; Vasas, A.; Hohmann, J. 14-Noreudesmanes and a phenylpropane heterodimer from sea buckthorn berry inhibit Herpes simplex type 2 virus replication. Tetrahedron. 2019, 75, 1364–1370.

66. Zhang, L.B.; Liao, H.B.; Zhu, H.Y.; Yu, M.H.; Lei, C.; Hou, A.J. Antiviral clerodane diterpenoids from Dodonaea viscosa. Tetrahedron. 2017, 72, 8036–8041.

67. Soares, A.R.; Abrantes, J.L.; Lopes Souza, T.M.; Leite Fontes, C.F.; Pereira, R.C.; de Palmer Paixão Frugulheti, I.C.; Teixeira, V.L. In vitro antiviral effect of meroditerpenes isolated from the Brazilian seaweed Styphopodium zonale (Dictyotales). Planta Med. 2007, 73, 1221–1224.

68. Krawczyk, E.; Łuczak, M.; Kniotek, M.; Nowaczyk, M. Cytotoxic, antiviral (in-vitro and in-vivo), immunomodulatory activity and influence on mitotic divisions of three taxol derivatives: 10-Deacetylbaccatin III, methyl (N-benzylo-(2′R,3′S)-3′-phenylisoserine) and N-benzylo-(2′R,3′S)-3′-phenylisoserine. J. Pharm. Pharmacol. 2005, 57, 791–797.

69. Wiart, C.; Kumar, K.; Yusof, M.Y.; Hamimah, H.; Fauzi, Z.M.; Sulaiman, M. Antiviral properties of ent-lebdenene diterpenes of Andrographis paniculata noes, inhibitors of herpes simplex virus type 1. Phytother. Res. 2005, 19, 1069–1070.

70. Barbosa, J.P.; Pereira, R.C.; Abrantes, J.L.; Cirne dos Santos, C.C.; Rebelo, M.A.; Frugulheti, I.C.; Teixeira, V.L. In vitro antiviral diterpenes from the Brazilian brown alga Dictyota paffii. Planta Med. 2004, 70, 856–860.

71. Isaka, M.; Chinthanom, P.; Srichomthong, K.; Thummarukcharoen, T. Lanostane triterpenoids from fruiting bodies of the bracket fungus Fomitopsis fei. Tetrahedron Lett. 2017, 58, 1758–1761.

72. Lv, X.J.; Li, Y.; Ma, S.G.; Qu, J.; Liu, Y.B.; Li, Y.H.; Zhang, D.; Li, L.; Yu, S.S. Antiviral Triterpenes from the Twigs and Leaves of Lynnaea ovariifolia. J. Nat. Prod. 2016, 79, 2824–2837.

73. Hassan, S.T.S.; Berchová-Bímová, K.; Petraš, J.; Hassan, K.T.S. Curcubitacin B interacts synergistically with antibiotics against Staphylococcus aureus clinical isolates and exhibits antiviral activity against HSV-1. S. Afr. J. Bot. 2017, 108, 90–94.

74. da Rosa Guimarães, T.; Quiroz, C.G.; Borges, C.R.; de Oliveira, S.Q.; de Almeida, M.T.; Bianco, É.M.; Moritz, M.I.; Carraro, J.L.; Palermo, J.A.; Cabrera, G.; Schenkel, E.P.; Reginatto, F.H.; Simões, C.M. Anti HSV-1 activity of halistanol sulfate and halistanol sulfate C isolated from Brazilian marine sponge Petromica citrina (Demospongiae). Mar. Drugs. 2013, 11, 4176–4192.

75. Laconi, S.; Madeddu, M.A.; Pompei, R. Autophagy activation and antiviral activity by a licorice triterpene. Phytother. Res. 2014, 28, 1890–1892.

76. Ikeda, T.; Yokomizo, K.; Okawa, M.; Tsuchihashi, R.; Kinjo J.; Nohara, T.; Uyeda, M. Anti-herpes virus type 1 activity of oleanane-type triterpenoids. Biol. Pharm. Bull. 2005, 28, 1779–1781.

77. Li, Y.; Jiang, R.; Ooi, L.S.; But, P.P.; Ooi, V.E. Antiviral triterpenoids from the medicinal plant Schefflera hexaphylla. Phytother. Res. 2007, 21, 466–470.

78. Mukherjee, H.; Ojha, D.; Bag, P.; Chandel, H.S.; Bhattacharyya, S.; Chatterjee, T.K.; Mukherjee, P.K.; Chakraborti, S.; Chattopadhyay, D. Anti-herpes virus activities of Achyranthes aspera: an Indian ethnomedicine, and its triterpene acid. Microbiol. Res. 2013, 168, 238–244.

79. Zhou, M.; Xu, M.; Ma, X.X.; Zheng, K.; Yang, K.; Yang, C.R.; Wang, Y.F.; Zhang, Y.J. Antiviral triterpenoid saponins from the roots of Ilex asperlla. Planta Med. 2012, 78, 1702–1705.
80. Liu, F.; Wang, Y.-N.; Li, Y.; Ma, S.-G.; Qu, J.; Liu, Y.-B.; Niu, C.-S.; Tang, Z. H.; Li, Y.-H.; Li, L.; Yu, S.-S. Triterpenoids from the twigs and leaves of *Rhododendron latoucheae* by HPLC–MS–SPE–NMR. *Tetrahedron.* 2019, 75, 296–307.

81. Sun, Y.L.; Wang, J.; Wang, Y.F.; Zhang, X.Y.; Nong, X.H.; Chen, M.Y.; Xu, X.; Qi, S.H. Cytotoxic and Antiviral Tetramic Acid Derivatives from the Deep-Sea-Derived Fungus *Trichobotrys effuse* DFFSCS021. *Tetrahedron.* 2015, 71, 9328–9332.

Álvarez, Á.L.; Habtemariam, S.; Abdel Moneim, A.E.; Melón, S.; Dalton, K.P.; Parra, F. A spiroketal-enol ether derivative from *Tanacetum vulgare* selectively inhibits HSV-1 and HSV-2 glycoprotein accumulation in Vero cells. *Antiviral Res.* 2015, 119, 8–18.

83. Pongmuangmul, S.; Phumiamorn, S.; Sanguansermsri, P.; Wongkattiya, N.; Fraser, I.H.; Sanguansermsri, D. Anti-herpes simplex virus activities of monogalactosyl diglyceride and digalactosyl diglyceride from *Clinacanthus nutans*, a traditional Thai herbal medicine. *Asian Pac. J. Trop. Biomed.* 2016, 6, 192–197.

84. Ma, F.W.; Kong, S.Y.; Tan, H.S.; Wu, R.; Xia, B.; Zhou, Y.; Xu, H.X. Structural characterization and antiviral effect of a novel polysaccharide PSP-2B from *Prunella spica*. *Carbohydr. Polym.* 2016, 152, 699–709.

85. Jin, F.; Zhuo, C.; He, Z.; Wang, H.; Liu, W.; Zhang, R.; Wang, Y. Anti-herpes simplex virus activity of polysaccharides from *Eucheuma gelatinae*. *World J. Microbiol. Biotechnol.* 2015, 31, 453–460.

86. Sahera, F.M.; Mohsen, M.S.A.; El-Sayed, O.H. Chemical structure and antiviral activity of sulfated polysaccharides from *Sargassum latifolium*. Conference: Medical Research Day, Faculty of Medicine, Jazan University, 2011.

87. Zhu, W.; Chiu, L.C.; Ooi, V.E.; Chan, P.K.; Ang, P.O. Jr. Antiviral property and mechanisms of a sulfated polysaccharide from the brown alga *Sargassum patens* against Herpes simplex virus type 1. *Phytomedicine.* 2006, 13, 695–701.

88. Lee, J.-B.; Takeshita, A.; Hayashi, K.; Hayashi, T. Structures and antiviral activities of polysaccharides from *Sargassum trichophyllum*. *Carbohydr. Polym.* 2001, 86, 995–999.

89. Bedoux, G.; Caama-Fuentes, E.; Boulho, R.; Marty, C.; Bourougoun, N.; Freile-Pelegryn, Y.; Robledo, D. Antiviral and Cytotoxic Activities of Polysaccharides Extracted from Four Tropical Seaweed Species. *Nat. Prod. Commun.* 2017, 12, 807–811.

90. Hardouin, K.; Bedoux, G.; Burlot, A.-S.; Donnay-Morenco, C.; Bergé, J.-P.; Nyvall Collen, N.; Bourougoun, N. Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae). *Algal Res.* 2016, 16, 233–239.

91. Vanderleli, E.; Eloy, Y.; de Araújo, I.; Quinderé, A., Fontes, B.; Mendes, G.; Cavalcanti, J.; Romanos, M.; Benevides, N. Structural features, molecular weight and anti-HSV activity of sulfated polysaccharides from three red seaweeds. *J. Chem. Pharm. Res.* 2016, 8, 164–170.

92. Bouthal, R.; Haslin, C.; Cherermann, J.-C.; Colliec-Jouault, S., Sinquin, C., Simon, G.; Cerantola, S.; Riadi, H.; Bourougoun, N. Antiviral Activities of Sulfated Polysaccharides Isolated from *Sphaerococcus coronopifolius* (Rhodophyta, Gigartinales) and *Boergesenia thauoides* (Rhodophyta, Ceramiales). *Marine Drugs.* 2011, 9, 1187–1209.

93. Saha, S.; Navidb. M. H.; Bandypahayay, S.S.; Schitzlerb, P.; Ray, B. Sulfated polysaccharides from *Laminaria angustata*: Structural features and in vitro antiviral activities. *Carbohydr. Polym.* 2012, 87, 123–130.

94. Lopes, N.; Ray, S.; Espada, S.F.; Bomfim, W.A.; Ray, B.; Faccin-Galhardi, L.C.; Linhares, R.E.C.; Nozawa, C. Green seaweed *Enteromorpha compressa* (Chlorophyta, Ulvaceae) derived sulfated polysaccharides inhibit herpes simplex virus. *Int. J. Biol. Macromol.* 2017, 102, 605–612.

95. Karmakar, P.; Pujol, C.A.; Damonte, E.B.; Ghosh, T.; Ray, B. Polysaccharides from *Padina tetrasirotmata*: Structural features, chemical modification and antiviral activity. *Carbohydr. Polym.* 2010, 80, 513–520.

96. Adhikari, U.; Mateu, C.G.; Chattopadhyay, K.; Pujol, C.A.; Damonte, E.B.; Ray, B. Structure and antiviral activity of sulfated fucans from *Stoechospernum marginatum*. *Phytochemistry.* 2006, 67, 2474–2482.

97. Mandal, P.; Mateu, C.G.; Chattopadhyay, K.; Pujol, C.A.; Damonte, E.B.; Ray, B. Structural features and antiviral activity of sulfated fucans from the brown seaweed *Cystoseira indica*. *Antivir. Chem. Chemother.* 2007, 18, 153–162.

98. Lee, J.B.; Hayashi, K.; Hashimoto, M.; Nakano, T.; Hayashi, T. Novel antiviral fuscoian from sporophyll of *Undaria pinnatifida* (Mekabu). *Chem. Pharm. Bull.* 2004, 52, 1091–1094.

99. Chattopadhyay, K.; Mateu, C. G; Mandal, P.; Pujol, C.A.; Damonte, E.B.; Ray, B. Galactan sulfate of *Grateloupia indica*: Isolation, structural features and antiviral activity. *Phytochemistry.* 2007, 68, 1428–1435.
100. Matsuhiro, B.; Conte, A.F.; Damonte, E.B.; Kolender, A.A.; Matulewicz, M.C.; Mejias, E.G.; Pujol, C.A.; Zúñiga, E.A. Structural analysis and antiviral activity of a sulfated galactan from the red seaweed Schizymenia binderi (Gigartinales, Rhodophyta). Carbohydr. Res. 2005, 340, 2392–2402.

101. Carlucci, M.J.; Pujol, C.A.; Ciancia, M.; Noseda, M.D.; Matulewicz, M.C.; Damonte, E.B.; Cerezo, A.S. Antitherpeic and anticoagulant properties of carrageenans from the red seaweed Gigartina skottsbergii and their cyclized derivatives: correlation between structure and biological activity. Int. J. Biol. Macromol. 1997, 20, 97–105.

102. Li, Z.; Liu, J. Zhao Y. Possible mechanism underlying the antitherpeic activity of a proteoglycan isolated from the mycelia of Ganoderma lucidum in vitro. J. Biochem. Mol. Biol. 2005, 38, 34–40.

103. Dong, C.X.; Hayashi, K.; Lee, J.B.; Hayashi, T. Characterization of structures and antiviral effects of polysaccharides from Porphyra tenera L. Chem. Pharm. Bull. 2010, 58, 507–510.

104. Lopes, N.; Faccin-Galhardi, L.C.; Espada, S.F.; Pacheco, A.C.; Ricardoy, N.M.; Linhares, R.E.; Nozawa, C. Sulfated polysaccharide of Caesalpinia ferrea inhibits herpes simplex virus and poliovirus. Int. J. Biol. Macromol. 2013, 60, 93–99.

105. Lee, J.B.; Tanihaka, T.; Hayashi, K.; Asagi, M.; Kasahara, Y.; Hayashi, T. Characterization and biological effects of two polysaccharides isolated from Acanthopanax sciadophylloides. Carbohydr. Polym. 2015, 116, 159–166.

106. Kanekiyo K.; Lee, J.B.; Hayashi, K.; Takenaka, H.; Hayakawa, Y.; Endo, S.; Hayashi, T. Isolation of an antiviral polysaccharide, nostoflan, from a terrestrial cyanobacterium, Nostoc flagelliforme. J. Nat. Prod. 2005, 68, 1037–1041.

107. Ghosh, P.; Adhikari, U.; Ghosal, P.K.; Pujol, C.A.; Carlucci, M.J.; Damonte, E.B.; Ray, B. In vitro anti-herpeic activity of sulfated polysaccharide fractions from Caulerpa racemosa. Phytochemistry. 2004, 65, 3151–3157.

108. Cavicchioli, V.Q.; Carvalho, O.V.; Paiva, J.C.; Todorov, S.D.; Silva Júnior, A.; Nero, L.A. Inhibition of herpes simplex virus 1 (HSV-1) and poliovirus (PV-1) by bacteriocins from Lactococcus lactis subsp. lactis and Enterococcus durans strains isolated from goat milk. Int. J. Antimicrob. Agents. 2018, 51, 33–37.

109. Quintana, V.M.; Torres, N.I.; Wachsmann, M.B.; Sinko, P.J.; Castilla, V.; Chikindas, M. Antitherpeic simplex virus type 2 activity of the antimicrobial peptide subtilosin. J. Appl. Microbiol. 2014, 117, 1253–1259.

110. Liang, X.; Nong, X.H.; Huang, Z.H.; Qi, S.H. Antifungal and Antiviral Cyclic Peptides from the Deep-Sea-Derived Fungus Simplicillium obtusatum EI0DSF 020. J. Agric. Food Chem. 2017, 65, 5114–5121.

111. Xuan Ma, Xu-Hua Nong, Zhe Ren, Jie Wang, Xiao Liang, Lu Wang, Shu-Hua Qi. Antiviral peptides from marine gorgonian-derived fungus Aspergillus sp. SC510. Tetrahedron Lett. 2017, 58, 1151–1155.

112. Gong, M.; Piraino, F.; Yan, N.; Zhang, J.; Xia, M.; Ma, J.; Cheng, J.; Liu, X. Purification, partial characterization and molecular cloning of the novel antiviral protein RC28. Peptides. 2009, 30, 654–659.

113. Vilas Boas, L.C.P.; de Lima, L.M.P.; Migliolo, L.; Mendes, G.d.S.; de Jesus, M.G.; Franco, O.L.; Silva, P.A. Linear antimicrobial peptides with activity against herpes simplex virus 1 and Aichi virus. Biopolym. 2017, 108, e22871.

114. El-Fakhrarany, E.M.; Uversky, V.N.; Redwan, E.M. Comparative Analysis of the Antiviral Activity of Camel, Bovine, and Human Lactoperoxidases Against Herpes Simplex Virus Type 1. Appl. Biochem. Biotechnol. 2017, 182, 294–310.

115. Levendosky, K.; Mizzenina, O.; Martinelli, E.; Jean-Pierre, N.; Kizima, L.; Rodriguez, A.; Kleinbeck, K.; Bonnaire, T.; Robbiani, M.; Zydowsky, T.M.; O’Keefe, B.R.; Fernández-Romero, J.A. Griffithsin and carrageenan combination to target herpes simplex virus 2 and human papillomavirus. Antimicrob. Agents Chemother. 2015, 59, 7290–7296.

116. Alboli Matanic, V.C.; Castilla, V. Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. Int. J. Antimicrob. Agents. 2004, 23, 382–389.

117. Waxman, L.; Darke, P.L. The herpesvirus proteases as targets for antiviral chemotherapy. Antivir. Chem. Chemother. 2000, 11, 1–22.

118. Reardan, J.E. Herpes simplex virus type 1 DNA polymerase. Mechanism-based affinity chromatography. J. Biol. Chem. 1990, 265, 7112–7115.

119. Valencia, F.; Veselemenak, R.L.; Bourne, N. In vivo evaluation of antiviral efficacy against genital herpes using mouse and guinea pig models. Methods Mol. Biol. 2013, 1030, 315–26.

120. Osada, N.; Kohara, A.; Yamaji, T.; Hirayama, N.; Kasai, F.; Sekizuka, T.; Kuroda, M.; Hanada, K. The genome landscape of the african green monkey kidney-derived Vero cell line. DNA Res. 2014, 21, 673–683.
121. D’Aiuto, L.; Williamson, K.; Dimitrion, P.; McNulty, J.; Brown, C.E.; Dokuburra, C.B.; Nielsen, A.J.; Lin, W.J.; Piazza, P.; Schurdak, M.E.; Wood, J.; Yolken, R.H.; Kinchington, P.R.; Bloom, D.C.; Nimgaonkar, V.L. Comparison of three cell-based drug screening platforms for HSV-1 infection. *Antiviral Res.* **2017**, *142*, 136–140.

122. Cotarelo, M.; Catalán, P.; Sánchez-Carrillo, C.; Menasalvas, A.; Cercenado, E.; Tenorio, A.; Bouza, E. Cytopathic effect inhibition assay for determining the in-vitro susceptibility of herpes simplex virus to antiviral agents. *J. Antimicrob. Chemother.* **1999**, *44*, 705–708.

123. Thi, T.N.; Deback, C.; Malet, I.; Bonnafous, P.; Ait-Arkoub, Z.; Agut, H. Rapid determination of antiviral drug susceptibility of herpes simplex virus types 1 and 2 by real-time PCR. *Antiviral Res.* **2006**, *69*, 152–157.

124. McClain, D.S.; Fuller, A.O. Cell-specific kinetics and efficiency of herpes simplex virus type 1 entry are determined by two distinct phases of attachment. *Virology.* **1994**, *198*, 690–702.

125. Silva, I.T.; Costa, G.M.; Stoco, P.H.; Schenkel, E.P.; Reginatto, F.H.; Simões, C.M.O. In vitro antiherpetic effects of a c-glycosylflavonoid enriched fraction of *Cecropia glaziouii* Sneth. *Lett. Appl. Microbiol.* **2010**, *51*, 143–148.

126. Klysik, K.; Pietraszek, A.; Karewicz, A.; Nowakowska, M. Acyclovir in the Treatment of Herpes Viruses – a Review. *Curr. Med. Chem.* **2018**, *25*, 233–243. doi: 10.2174/0929867325666180309105519.

127. Ouyang, J.; Sun, F.; Feng, W.; Xie, Y.; Ren, L.; Chen, Y. Antimicrobial Activity of Galangin and Its Effects on Murein Hydrolases of Vancomycin-Intermediate *Staphylococcus aureus* (VISA) Strain Mu50. *Chemother.* **2018**, *63*, 20.

128. Céliz, G.; Daz, M.; Audisio, M.C. Antibacterial activity of naringin derivatives against pathogenic strains. *J. Appl. Microbiol.* **2011**, *111*, 731.

129. Pujol, C.A.; Carlucci, M.J.; Matulewicz, M.C.; Damonte, E.B. Natural sulfated polysaccharides for the prevention and control of viral infections. *Topics in Heterocyclic Chemistry.* **2007**, *11*, 259–281. Springer, Berlin, Heidelberg.

130. Choi, J.H.; Jang, A.Y.; Lin, S.; Lim, S.; Kim, D.; Park, K.; Han, S.M.; Yeo, J.H.; Seo, H.S. Melittin, a honeybee venom-derived antimicrobial peptide, may target methicillin-resistant *Staphylococcus aureus*. *Mol. Med. Rep.* **2015**, *12*, 6483.

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