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# Review on the Antiviral Organic Agents against Fish Rhabdoviruses

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**Abstract:** Fish rhabdoviruses are harmful single-stranded RNA viruses with high mortality rates which cause considerable economic losses in aquaculture. It is imperative to explore and develop new antiviral compounds against them. In recent years, in addition to inorganic antiviral substances, more than 50 different organic compounds have been confirmed to be effective in the prevention and treatment of rhabdovirus infection and its dissemination in fish. The main types of extracts or agents and their trial designs are here considered for review. This review reveals the reported antiviral activities of extracts from organisms, proteins, lipids, polysaccharides, nucleic acids, coumarin derivatives, arctigenin derivatives, and other antiviral organic molecules against fish rhabdoviruses, respectively. Additionally, their antiviral mechanisms of action include direct virucidal effects, inhibiting virus-induced host cell apoptosis, the blocking of the viral replication cycle, affecting gene expression and innate antiviral immune responses, and so on. This review also gives perspectives on how to comprehensively explore the potential applications of the candidate molecules, which lay the foundation for the future development of new compounds or strategies for the prevention and control of fish rhabdoviruses in aquaculture.

**Keywords:** fish rhabdoviruses; antiviral agents; antiviral mechanisms; virus replication



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## 1. Introduction

The need for food, including protein sources, will increase considerably in the coming years due to an increasing global population and urbanization and changing eating habits [1,2]. Demand for aquaculture is estimated to reach 62% of total global production by 2030 [3]. In this context, the growth of aquaculture in terms of providing a protein source for human consumption has enormous potential. It can also be considered a sustainable food industry. However, with the expansion of marine and freshwater aquaculture, outbreaks of viral diseases have been frequently reported and increasingly become the significant threats to the healthy development of aquaculture [4].

As a variety of highly invasive viral pathogens, fish rhabdoviruses are negative single-stranded RNA viruses belonging to the members of the family Rhabdoviridae, which can infect a wide range of host species, resulting high economic losses. Affected fish present a destruction of tissues in the kidney, spleen, and liver, leading to hemorrhage, loss of water–salt balance, and the impairment of the immune response. To date, more than 30 different strains of rhabdoviruses have been isolated and identified from wild or cultured fish, including viral hemorrhagic septicemia virus (VHSV), infectious hemorrhagic necrosis virus (IHNV), spring viremia of carp virus (SVCV), *Micropterus* salmoides rhabdovirus (MSRV), and hirame rhabdovirus (HIRRV). Particularly IHNV, VHSV and SVCV, cause significant morbidity and mortality in wild and farmed fish, with mortality rates reaching up to 100% in certain disease outbreaks. As examples, epizootic outbreaks of IHNV in Atlantic salmon from 1992 to 1996 and from 2001 to 2003 resulted in estimated economic losses of $40 million dollars in inventory, which represented approximately $200 million

dollars in lost sales. Due to viral hemorrhagic septicemia (VHS), European fish farmers estimated losses of approximately £40 million pounds in 1991. Thus, high mortality and transmission represent a severe threat to aquaculture due to the lack of approved antiviral treatments [5]. Researchers have made great efforts in molecular sequencing and the phylogenetic analysis of different strains, as well as the susceptibility and pathology of fish hosts [6–8]. Basic theoretical studies on the pathogenic processes and the immunological and cellular modulation of fish rhabdoviruses have also been performed [9–12]. These reports provided solid data for the potential development of antiviral techniques or drugs in the future.

Given the devastating damage to aquatic rhabdoviruses in aquaculture, there is a strong demand for developing antiviral measures to prevent the infection of fish rhabdoviruses. Since the 1990s, scientists have begun to explore antiviral actions for fish rhabdoviruses. The earliest study from PubMed was carried out by Batts et al. (1991) [13], who first used a type of inorganic molecule-low levels of iodine-to inactivate IHNV and block transmission to the water supplies of hatcheries. Over the last decade, many antiviral agents, including inorganic chemical disinfectants, antibiotics, and various types of vaccines against specific strains of fish rhabdoviruses, have been produced for their antiviral activities [14–17]. However, to date, only one reported commercially approved vaccine named APEX-IHN®, is available against IHNV in aquaculture [18], given the real multiple-factor water environment, complicated rhabdoviruses–host relationships, and the high cost of vaccines. Moreover, inorganic chemicals and antibiotic application have been restricted globally against aquatic disease outbreaks due to their apparent accumulation in the tissues, which enables the development of resistant bacteria [19]. Hence, the search for alternative, environmentally friendly and effective disinfecting agents against fish rhabdoviruses has garnered interest in the last few years.

In fact, in addition to the above-mentioned iodine solution and recently reported anti-rhabdovirus substance, red elemental selenium ($Se^0$) [20], many other multi-sourced antiviral agents which are organic chemicals have exhibited considerable potential in preventing the infection of rhabdoviruses in fish. For example, several extracts from different herbal and medicinal plants have been tested to combat fish rhabdoviruses in aquaculture and were found to be as safe and eco-friendly substances that modulate immune status, enhance growth performance, and prevent the viral infection of aquaculture animals [19]. Furthermore, active organic ingredients or compounds with defined molecular structures have also been used to defend aquaculture animals from rhabdoviruses attacks. Therefore, the objective of this review was to survey organic molecules which display activity against fish rhabdoviruses. The review will primarily focus on extract mixtures, proteins, nucleotide acids, lipids, polysaccharides, several derivatives, and other antiviral organic agents that act against fish rhabdoviruses, as summarized in Tables 1–7. Research into antiviral properties, antiviral mechanisms, and the trial designs of these organic substances will be discussed in detail.

## 2. Antiviral Organic Agents against Fish Rhabdoviruses

### *2.1. Anti-Fish Rhabdovirus Mixtures Extracted from Different Organisms*

To search for pharmacological substances of different origins that are effective against fish rhabdoviruses, researchers have extracted several mixtures from marine microalgae, brown alga (*Eisenia bicyclis*), red alga (*Polysiphonia morrowii*), algal (*Ecklonia cava*), fungus (*Bacillus subtilis*), and herbal or plants, such as the *Celosia cristata* and *Raphanus sativus*, olive leaf, *Cassia alata*, and *Phyllanthus acidus* [21–28] (Table 1). These extracts have been applied to treat cultured fish cells (in vitro) or inject fish (in vivo) in order to detect their anti-VHSV or anti-IHNV activities, respectively. The results demonstrated that only the substances extracted from *Eisenia bicyclis* possessed the ability to directly inactivate viral particles [21]. Other extracts were only able to reduce the cytopathic effects, inhibit virus replication, and lead to the decrease of progeny virus titers in treated cell lines or fish bodies [22–24]. Extracts from algal *Ecklonia cava* were able to enhance antiviral in vivo

immune response against VHSV [25]. Unexpectedly, although the extract mixture from *Celosia cristata* and *Raphanus sativus* roots had no effect when added after viral inoculation in fish, it was able to induce the gene expression involved in the innate immune response, which might mediate the antiviral activity of the extracts against VHSV [26]. Recently, two organic mixtures extracted from olive leaf and *Cassia alata* and *Phyllanthus acidus*, were confirmed to inhibit cell-to-cell membranes and virus absorption against VHSV and IHNV in vitro [27,28], respectively. Future work should examine the determining compositions and antiviral ingredients in the above-described mixture extracts.

**Table 1.** Extracts against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| IHNV | In vitro | Extract from marine brown alga, *Eisenia bicyclis* | Direct inactivation of the viral particles | [21] |
| VHSV | In vitro | Extracts from Marine microalgae | Inhibit virus replication | [22] |
| IHNV | In vitro | Extract from red alga, *Polysiphonia morrowii* | Reduce virus-induced cytopathic effect | [23] |
| VHSV | In vitro, in vivo | Extract from *Bacillus subtilis* | Inhibit virus infection/reduce cytopathic effect (CPE) | [24] |
| VHSV | In vitro, in vivo | Extract from algal *Ecklonia cava* | Enhance antiviral immune response | [25] |
| VHSV | In vitro, in vivo | Extracts from *Celosia cristata* and *Raphanus sativus* roots | Reduce virus titer/ induce gene expression involved in the innate immune response | [26] |
| VHSV | In vitro | Olive leaf extract | Inhibit cell-to-cell membrane fusion and decrease virus titers and viral protein accumulation | [27] |
| IHNV | In vitro | Extracts from *Cassia alata* and *Phyllanthus acidus* | Reduce the plaques and inhibit virus absorption | [28] |

### 2.2. Antiviral Proteins

As the hosts' first line of defense during viral infection, interferons (IFN) are rapidly synthesized to combat the invading pathogens. The secretion of IFN can trigger the upregulation of downstream genes responsible for inhibiting viral replication or degrading viral components, including protein kinase R (PKR), oligoadenylate synthetases (OAS), and myxovirus resistance (Mx) proteins and so on [29]. To date, Saint-Jean et al. (2006) [30] have confirmed IFN-mediated antiviral activity against IHNV in several cell lines. In higher vertebrates, Tank-binding kinase 1 (TBK1) has demonstrated its pivotal role in the induction of type I IFNs. In 2017, Yan et al. [31] further confirmed its ability in the antiviral innate immune response of black carp against SVCV. Furthermore, Chico et al. (2019) [32] identified an IFN-induced protein with tetratricopeptide repeats 5 (IFIT5), which confirmed the participation in rainbow trout's nucleated red blood cell (RBCs) antiviral immune response to VHSV. A correlation exists between the highest IFIT5 expression levels and the decline in VHSV replication after exposure for 6 h in RBCs. Recently, Chico et al. (2021) [33] applied the immunoprecipitated proteome technique to analyze the RBSc exposed to VHSV and identified another peroxiredoxin protein, natural killer enhancing factor (NKEF), whose increased expression correlated with decreased VHSV replication in RBCs. They further found a correlation between silencing of the *nkef* gene and a decrease in the face of genes related to the IFN1 pathway, which indicates that NKEF is involved in the antiviral mechanisms of rainbow trout RBCs against VHSV. Similarly, another IFN-induced endoplasmic reticulum-associated protein, viper in (known as radical S-adenosyl methionine domain containing 2, RSAD2) from rockfish, was characterized for its antiviral function [34].

Moreover, Mx proteins are well-known antiviral gatekeepers that restrain the negative-stranded RNA viruses, and the expression of the Mx genes is controlled by IFNs [35]. In aquaculture, Caipang et al. (2003) [36] found that the *Paralichthys olivaceus* Mx was able to inhibit VHSV/HIRRV replication by blocking the transcription of viral subgenomic mRNAs. Moreover, one interesting study demonstrated that three Mx isoforms of the gilthead seabream (*Sparus aurata*) did not the possess synergistic effects of antiviral activity, and instead displayed a harmful interference against VHSV [37].

Except for the IFN and Mx, another three fish proteins or peptides, including fathead carp cytidine/uridine monophosphate kinase 2 (CMPK2), spotted scat hepcidin, and turbot NK-Lysin peptide, also exerted their antiviral potentials in response to viral infection [38–40] (Table 2), respectively. In addition, recent work has also demonstrated that C-reactive protein-like protein (CRP1-7, CRPs) from zebrafish could reduce autophagy activity by initially disturbing the cholesterol ratios in the host cellular membranes [41], which in turn negatively affects the intracellular regulation of reactive oxygen species (ROS) and increases lysosomal pH as a consequence. Such pH changes exerted an inhibitory direct effect on SVCV replication by disrupting the pH-dependent membrane-fusogenic ability of the viral glycoprotein. Additionally, another zebrafish protein, G protein-coupled bile acids receptor 1 (GPBAR1), similarly possessed an antiviral response, the overexpression of which significantly inhibited SVCV infection in the zebrafish cell line ZF4 with bile acid (INT777) treatment [42].

Interestingly, the other two peptides—human neutrophil peptide 1 (HNP1) and milk-derived proteins—designed as bovine caseins were able to elicit strong antiviral responses and reduce the yield of infectious progeny particles, which provided convincing evidence in support of a role for non-fish factors as suitable candidates to prevent viral infections in fish [43,44].

Furthermore, as a well-characterized pore-forming lytic amphiphilic peptide, melittin is susceptible to being used as a vehicle in lipid membranes and has been utilized to study their antiviral properties. Falco et al. [45] performed an assay based on melittin- loaded immunoliposomes using monoclonal antibodies targeting the glycoprotein of VHSV. This study confirmed the antiviral action of these immunoliposomes via the direct inactivation of the virus in vitro. It is one of the few reported molecules that can directly inactivate the virus and bear their antiviral activity after treatment, which is obviously different from most other antiviral chemicals able to act against fish rhabdoviruses.

**Table 2.** Antiviral proteins against fish rhabdoviruses and their antiviral effects.

| Virus Strain. | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| IHNV | In vitro | Fish interferon | IFN-induced cytokines | [30] |
| SVCV | In vitro | Black carp TBK1 | Involve in the antiviral innate immune response | [31] |
| VHSV | In vitro | Rainbow trout IFIT5 | Implicate in the antiviral immune response of RBCs | [32] |
| VHSV | In vitro | Rainbow trout NKEF | Involve in the antiviral immune response of RBCs | [33] |
| VHSV | In vitro | Rockfish viperin | Enervate virus transcription and replication | [34] |
| VHSV/HIRRV | In vitro | Japanese flounder Mx | Block the transcription of viral subgenomic mRNAs | [36] |
| VHSV | In vitro | Gilthead seabream Mx isoforms | Inhibit virus replication but no synergistic effects | [37] |
| SVCV | In vitro, in vivo | Carp CMPK2 | IFN-stimulated gene against SVCV infection | [38] |
| SCRV | In vitro | Spotted scat hepcidin | Reduce cytopathic effect | [39] |
| SVCV | In vitro | Turbot NK-Lysin Peptide | Inhibit viral particles binding, the fusion of virus and cell membranes | [40] |
| SVCV | In vivo | Zebrafish CRP1-7 | Block autophagy through interactions with cell membrane cholesterol | [41] |

**Table 2.** *Cont.*

| Virus Strain. | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| SVCV | In vitro | Zebrafish GPBAR1 | Activate RLR signaling pathway and induce the production of ISGs | [42] |
| VHSV | In vitro | Human neutrophil peptide 1 (HNP1) | Interfere with G protein-dependent fusion/ up-regulate genes related to the IFN response | [43] |
| IHNV | In vitro, in vivo | Bovine caseins | Reduce the infective titer | [44] |
| VHSV | In vitro | Melittin loaded-immunoliposomes | Direct inactivation of the virus/ inhibit virus infectivity | [45] |
| VHSV | In vitro | Commercially available protease-Neutrase® | Reduce the virus titer | [46] |

### *2.3. Antiviral Nucleic Acids*

In recent years, two types of artificial nucleic acids were developed and used for inhibiting the growth of fish rhabdoviruses (Table 3). One is a RNA aptamer, which binds to a wide variety of targets with high affinity and specificity. This could be developed as an effective tool in pharmaceutical and antiviral research. In cultured hirame natural embryo (HINAE) cells, the screened HIRRV-specific and VHSV-specific RNA aptamers were applied to bind specifically to and inhibit the growth of fish rhabdoviruses, respectively [47,48]. The results suggested that RNA aptamers could reduce the appearance of cytopathic effects and might be a helpful tool for protection against fish rhabdoviruses.

The other type is small interfering RNAs (siRNAs), which are double-stranded RNA molecules capable of silencing the expression of a specific proteins triggering the RNA interference (RNAi) pathway. Our previous study evaluated the antiviral activities of four siRNAs corresponding to the *Siniperca chuatsi* rhabdovirus (SCRV) nucleoprotein (N) gene against the SCRV in the fish cell line *Epithelioma papulosum cyprini* (EPC) [49]. We screened an effective siRNA molecule specific to the SCRV *N* gene, which was able to significantly inhibit the expression of nucleoprotein of SCRV and led to the reduction of progeny virus titer. In recent years, the inhibition of nucleoprotein, phosphoprotein (P), and RNA-dependent RNA polymerase (L) gene expression by synthetic siRNA molecules was also able to reduce SVCV replication [50,51], further indicating that targeting fish rhabdoviruses genes using RNAi might help to control infection in fish (Table 3).

**Table 3.** Antiviral nucleic acids against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| HIRRV | In vitro | RNA aptamers | Decrease of cytopathic effect | [47] |
| VHSV | In vitro, in vivo | RNA aptamers | Decrease of cytopathic effect and growth | [48] |
| SCRV | In vitro | siRNAs to N gene | Inhibit *N* gene expression | [49] |
| SVCV | In vitro | siRNAs to N/P genes | Inhibit *N/P* gene expression | [50] |
| SVCV | In vitro | siRNAs to L gene | Inhibit *L* gene expression | [51] |

### *2.4. Antiviral Lipids and Polysaccharides*

Lipids are vital elements that affect the growth and immune functions of healthy fish. Lipid supplementation in the fish diet has been shown to enhance protection against several pathogens [52]. Recently, two lipid molecules—palmitic acid and α-Lipoic acid—have been investigated for the activities and mechanisms of anti-fish rhabdoviruses, respectively (Table 4). The results suggested that palmitic acid modulated SVCV infection in zebrafish and reduced mortality and viral titers, which seemed to be associated with the inhibition of autophagic flux and was independent of other immune processes [52]. α-Lipoic acid, a

potent antioxidant, has also been suggested to exhibit antiviral activity against VHSV [53] and could significantly increase the survival rate of the VHSV-infected largemouth bass in both through co-injection and post-injection methods. Differently to palmitic acid, α-Lipoic acid treatment could upregulate the expression of several antiviral genes, including the interferon regulatory factor 7 (*irf7*), *viperin*, and interferon-stimulated gene product 15 (*isg15*), demonstrating that the antiviral effect of α-Lipoic acid might be related to the immune response of fish. Similarly, as a family of cholesterol-oxygenated derivatives with diverse roles in many biological activities, oxysterols have been linked with the induction of a cellular antiviral state. Pereiro et al. (2017) [54] found that the in vivo overexpression of *ch25hb* (cholesterol 25-hydroxylase), the putative homolog of mammalian Ch25h in zebrafish larvae, was able to significantly reduce mortality after an SVCV challenge. The administration of 25-hydroxycholesterol (25HC) to the zebrafish cell line ZF4 also was also able to affect viral replication, which further confirmed the antiviral role of 25HC against fish rhabdoviruses.

In addition to the above lipid molecules, recent work has shown the anti-fish rhabdovirus activities of two polysaccharides [55,56]. One is a crude polysaccharide derived from seaweed (CSP), which possesses excellent safety and a good ability to inhibit IHNV and could be involved in preventing viral attachment and release [55]. The other is *Lactobacillus rhamnosus* GCC-3 exopolysaccharides (GCC-3 EPS). Xie et al. (2022) [56] found that supplementation of GCC-3 EPS significantly improved the survival rate of zebrafish and upregulated the expression of genes related to type I IFN antiviral immunity. In vitro experiments further confirmed its ability to inhibit SVCV replication in zebrafish ZF4 cell lines. These studies shed new light on developing novel agents against fish rhabdoviruses in aquaculture.

**Table 4.** Antiviral lipids and polysaccharides against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| SVCV | In vivo | Palmitic acid | Inhibit autophagic flux | [52] |
| VHSV | In vitro, in vivo | α-Lipoic acid | Promote upregulation of antiviral genes and suppress oxidative stress | [53] |
| SVCV | In vitro, in vivo | Zebrafish 25-hydroxycholesterol | Reduce mortality after virus challenge/inhibit viral replication | [54] |
| IHNV | In vitro | Crude polysaccharide derived from seaweed | Involve in preventing viral attachment and release | [55] |
| SVCV | In vitro, in vivo | Lactobacillus rhamnosus GCC-3 exopolysaccharides | Upregulate the expression of genes related to IFN antiviral immunity | [56] |

### *2.5. Coumarin Derivatives*

To date, coumarin derivatives are the most numerous types of organic chemicals that possess antiviral activity towards fish rhabdoviruses according to the reported kinds of literature (Table 5). As an essential oil extract from higher plants, such as *Rutaceae* and *Umbelliferae*, coumarin has been shown to have different biological properties, including antitumor, antimicrobial, antioxidant, and anti-inflammatory activities [57]. In aquaculture, recent studies indicated that the imidazole substituent groups played an essential role in decreasing fish rhabdovirus replication, and several imidazole coumarin derivatives demonstrated their antiviral abilities to aquatic rhabdoviruses (Table 5). The results indicated that most of the reported coumarin derivatives, for example, B4, C2, BBC, A9, C10, and D5 [58–63], were able to reduce apoptosis in virus-infected cells, enhance antioxidative gene expression or maintain antioxidant-oxidative balance and thus reduce the virus titers of infectious viruses. 7-[6-(2-methylimidazole) hexyloxy] coumarin (D5) was also able to repress SVCV viral glycoprotein gene expression and elicit an innate immune

response in vivo [64]. Mechanistically, D5 can disrupt viral binding to the cell surface or translocation to the cytosol and significantly suppress SVCV-activated protective autophagy during the early stages of viral infection [65]. Similar to the antiviral mechanism of D5, coumarin derivative C3007 is able to decrease SVCV gene expression and induce antiviral immunity in vitro and in vivo [66]. Another derivative, 7-(6-benzimidazole) coumarin (C10), can inhibit the IHNV adhesion ability, which is similar to D5 against SVCV [67]. Besides the decline of apoptosis in infected cells, a recent study indicated that the 7-(4-(4-methyl-imidazole))-coumarin (C2) is able to decrease the horizontal transmission, which is beneficial to antiviral activity against SVCV [68].

**Table 5.** Coumarin derivatives against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| SVCV | In vitro | imidazole coumarins (B4 and C2) | Decline cell apoptosis/enhance anti-oxidative gene expression | [58] |
| SVCV | In vitro, in vivo | 7-(4-benzimidazole-butoxy)-coumarin (BBC) | Decline cell apoptosis and recover caspases activity/up-regulate both antiviral responses and cellular IFN response | [59] [60] |
| IHNV | In vitro, in vivo | Coumarin derivative A9 | Block IHNV-induced apoptosis/repress IHNV gene expression | [61] |
| SVCV | In vitro, in vivo | 7-(6-benzimidazole) coumarin (C10) | Reduce apoptosis/maintain antioxidant-oxidant balance | [62] |
| IHNV | In vitro, in vivo | 7-[6-(2-methylimidazole) hexyloxy] coumarin (D5) | Decrease apoptosis/maintain antioxidant-oxidant balance | [63] |
| SVCV | In vivo | 7-[6-(2-methylimidazole) hexyloxy] coumarin (D5) | Repress viral glycoprotein gene expression/elicit innate immune response | [64] |
| SVCV | In vitro | 7-[6-(2-methylimidazole) hexyloxy] coumarin (D5) | Disrupt viral binding or translocation to cytosol/suppress autophagy | [65] |
| SVCV | In vitro, in vivo | Coumarin derivative (C3007) | Decrease SVCV gene expression/ induce antiviral immune | [66] |
| IHNV | In vitro, in vivo | 7-(6-benzimidazole) coumarin (C10) | Inhibit IHNV adhesion/decrease viral loads | [67] |
| SVCV | In vitro, in vivo | 7-(4-(4-methyl-imidazole))-coumarin (C2) | Decrease mortality/viral titers/ horizontal transmission | [68] |

### *2.6. Arctigenin Derivatives*

Arctigenin is a natural lignan compound extracted from *Arctium lappa*, which is able to regulate numerous intracellular activities, such as antioxidative, anti-inflammatory, and anticancer activities [69]. However, this material has the defect of low water solubility, and thus its derivatives or analogs have herein been designed and synthesized to overcome this disadvantage [70]. In aquaculture, several arctigenin derivatives have been synthesized and investigated for their antiviral activities. Similar to coumarin derivatives, the length of linker and imidazole substituent groups of arctigenin are two critical factors that inhibit the replication of fish rhabdoviruses. In Table 6, we listed the published arctigenin analogs with the antiviral activity of fish rhabdoviruses [71–74]. Mechanically, arctigenin derivatives can cause profound the inhibition of virus replication by inhibiting apoptosis in SVCV- or IHNV-infected cells and reducing viral gene expression in vitro and in vivo. In addition, Shen et al. (2020) [75] found that an arctigenin derivative, 4-(8-(2-bromoimidazole)octyloxy)-arctigenin (BOA), showed higher antiviral activities against three strains of aquatic rhabdoviruses (SVCV/IHNV/MSRV) in vitro, which suggests its broad spectrum against multiple rhabdoviruses. BOA was further discovered to not directly impact viral particles or interfere with SVCV adsorption but to reduce the release of viral particles at the late stage

of virus replication, demonstrating its essential role in the response to the viral replication cycle of fish rhabdoviruses [75].

**Table 6.** Arctigenin derivatives against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| SVCV | In vitro | Arctigenin derivatives | Inhibit SVCV-induced apoptosis and reactive oxygen species | [71] |
| IHNV | In vitro | 2Q and 6 A | Inhibit apoptosis/affect the early replication of IHNV | [72] |
| IHNV | In vitro, in vivo | Arctigenin derivative 15 | Inhibit cell apoptosis/elicit anti-inflammation response | [73] |
| SVCV | In vitro, in vivo | 4-(8-(2-ethylimidazole) octyloxy)-arctigenin (EOA) | Decrease the cytopathic effect/inhibit SVCV glycoprotein expression | [74] |
| IHNV/SVCV/MSRV | In vitro | 4-(2-methylimidazole) octanoxy-arctigenin (MON) | Inhibit cell apoptosis/reduce the release of viral particles | [75] |

### *2.7. Other Antiviral Ingredients*

In Table 7, we summarized the anti-fish rhabdoviruses organic chemicals that are not affiliated with the above-described types of molecules. Some of these are chemicals or drugs that have been used as model antibiotics in studies, such as ribavirin [76,77], mycophenolic acid [78], dexamethasone [79], anisomycin [80], or 8-hydroxyquinoline [81], which have been applied as immune inhibitors or anti-infective drugs for their definite pharmacological properties. In aquaculture research, these molecules have been used to treat rhabdovirus-infected cells or fish. Studies demonstrated that they are able to inhibit virus-induced apoptosis and decrease the viral gene expression, which thus reduces virus titers and achieves antiviral effects.

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione), the principal polyphenol isolated from turmeric and flavonoids isolated from *Rhus verniciflua* are both important organic chemical molecules extracted from medical plants [82]. They have exhibited many pharmacological effects, such as anti-inflammatory, antioxidant, anti-apoptotic, and so on. In the field of aquatic virology, Jeong et al. (2015) [83] and Kang et al. (2012) [84] confirmed the antiviral activity of these extracts against VHSV in vitro. Currently, the anti-VHSV mechanism of *Rhus verniciflua* flavonoids is still not clear, but the antiviral activity was certainly not associated with direct virucidal effects. On the other hand, curcumin can inhibit VHSV replication by suppressing viral entry via the rearrangement of the F-actin/G-actin ratio by downregulating the heat shock cognate protein 71 (HSC71) [83]. The comparative proteomic analysis showed that Hsc71 was differentially expressed after VHSV infection in fathead minnow cells (FHM), which is the primary protein interacting with fibronectin (FN)1, actins, and gelsolin (GSN), as a strong target candidate for the protection from VHSV infection at the viral entry stage. The downregulation of Hsc71 by curcumin treatment could be responsible for the antiviral activity of curcumin in FHM cells.

In addition, the anti-fish rhabdoviruses activities of other substances listed in Table 7, including rhodanine derivative (LJ001), phenylpropanoid derivative (E2), N,N′-disubstituted 2,5-piperazinediones, ursolic acid, bavachi, and β-glucan [85–90], have been reviewed in the published literature [91]. In general, rhodanine derivative (LJ001), phenylpropanoid derivative (E2), and N,N′-disubstituted 2,5-piperazinediones can block the viral entry (IHNV), post-entry transport (SVCV), and virus assembly/release (VHSV) after treatment in virus-infected cells [85–87], respectively, which impaired the viral replication cycle and exerted antiviral effects against fish rhabdoviruses. However, ursolic acid (3β-hydroxy-urs-12-en-28-oic-acid) isolated from *Prunella vulgaris L.* and bavachi utilize their antiviral ability against IHNV or SVCV by inhibiting viral gene expression [88,89]. At the same time,

β-glucan treatment can activate the IFN signal pathway and induce an innate immune response, which is beneficial to the antiviral immunity of SVCV-infected fish bodies [90]. Recently, another two small, natural molecules, dihydroartemisinin (DHA) and (S, S)-(+)-tetrandrine (TET), have also been investigated for their anti-SVCV activities [92]. Results indicated that DHA and TET might be able to stimulate the host's innate immune response to combat viral infection and may possess positive effects regarding the suppression of SVCV infection by interfering with early-stage viral replication, thus holding great potential as immunostimulants able to reduce the risk of fish rhabdovirus disease outbreaks.

**Table 7.** Other organic agents against fish rhabdoviruses and their antiviral effects.

| Virus Strain | Design of Trial | Antiviral Agent | Antiviral Effects | References |
|---|---|---|---|---|
| VHSV | In vitro | Ribavirin | Decrease viral transcription | [76] |
| IHNV | In vitro | Ribavirin | Inhibit apoptosis/damage to the viral particle of IHNV | [77] |
| VHSV | In vitro | Mycophenolic acid | Reduce virus titer/inhibit viral protein synthesis | [78] |
| VHSV | In vitro | Dexamethasone | Inhibit HSP90α expression | [79] |
| SVCV | In vitro, in vivo | Anisomycin (Ani) | Inhibit viral gene expression | [80] |
| MSRV | In vitro, in vivo | 8-hydroxyquinoline | Reduce the cytopathic effect/decrease viral nucleoprotein expression | [81] |
| VHSV | In vitro | Curcumin | Suppress viral entry via the rearrangement of the F-actin/G-actin ratio via downregulating HSC71 | [83] |
| IHNV/VHSV | In vitro | Flavonoids isolated from Rhus verniciflua | Not associated with direct virucidal effects | [84] |
| IHNV | In vitro, in vivo | Rhodanine derivative (LJ001) | Inhibit virus–cell membrane fusion during viral entry | [85] |
| SVCV | In vitro, in vivo | Phenylpropanoid derivative (E2) | Decline the apoptosis/block the post-entry transport process of the virus | [86] |
| VHSV | In vitro | *N*, *N*′-disubstituted 2,5-piperazinediones | Impair virus assembly/release | [87] |
| IHNV | In vitro, in vivo | Ursolic acid from *Prunella vulgaris* L. | Decrease the viral titer/inhibits viral gene expression | [88] |
| SVCV | In vitro | Bavachi | Block cell apoptosis/inhibit viral gene expression | [89] |
| SVCV | In vitro, in vivo | β-glucan | Activate IFN signal pathway and innate immune response | [90] |
| SVCV | In vitro, in vivo | Dihydroartemisinin (DHA) | Affect early-stage viral replication/ stimulate the host innate immune | [92] |
| SVCV | In vitro, in vivo | (S, S)-(+)-tetrandrine (TET) | Interfere with SVCV entry/stimulate the host innate immune response | [92] |

## 3. Perspectives and Outlook

In view of severe hemorrhagic septicemia and the massive numbers of deaths of freshwater and marine fish caused by rhabdoviruses every year, which resulted in significant economic losses, these viruses have gained much more attention, and researchers have begun to focus on the development of various antiviral measures and techniques. Over the last few years, a large number of diverse biological compounds have been extracted or synthesized, and identified for their antiviral abilities against several strains of fish rhabdoviruses. According to the difference in their biochemical properties, we here classified these organic chemicals as mixtures extracted from various organisms, antiviral proteins,

antiviral lipids, antiviral polysaccharides, antiviral nucleic acids, coumarin derivatives, arctigenin derivatives, and other antiviral molecules in Tables 1–7.

Of these agents, antiviral proteins and the derivatives of small, natural molecules (such as coumarin and arctigenin) account for the majority and antiviral lipids and polysaccharides reported against fish rhabdoviruses are lesser in number. The antiviral mechanisms of these chemicals were diverse. Still, among them, the percentage of antiviral compounds displaying direct virucidal effects was limited, and most of these generally inhibited progeny virus replication by blocking virus-induced host cell apoptosis; interfering with viral attachment, assembly or release; disrupting virus virus gene expression; activating IFN and innate antiviral immune responses; and so on. Often, the same compound exerted multiple antiviral mechanisms against fish rhabdoviruses. In turn, different compounds also demonstrated the same antiviral action in various studies. Additionally, several molecules, including imidazole coumarins B4 [58], coumarin derivative C3007 and C2 [66,68], Dihydroartemisinin (DHA), and (S, S)-(+)-tetrandrine (TET) [92], may disrupt horizontal viral transmission to some extent, suggesting that they could be applied not only as antiviral agents in aquatic systems but also as preventive measures against viral transmission in an aquaculture environment.

However, we should note that, apart from one commercially available protease-Neutrase® for the inactivation of VHSV and koi herpesvirus (KHV) [46], as seen in Table 2, other above-described anti-fish rhabdovirus agents are still not available for use in the aquaculture industry, despite having exhibited superior antiviral activities in laboratory research. Certain adverse factors might counteract their advantages and the efficacy of these compounds in practice, mainly manifesting as the following: (i) most antiviral chemicals are pure chemical molecules or organic extracts from organisms, and whether they can still bring to bear their antiviral roles in natural ponds or ocean ranches because of difficult time and space factors must be further confirmed; (ii) the investigation of in vivo antiviral effects of these agents were mostly carried out by the injection of fish bodies in laboratory conditions, and how to find a method of administration in real water environments is an urgent problem; and(iii) the cost of large-scale commercial production should be considered. Currently, treatment doses of different compounds are relatively low in lab situations, but whether the expenses of the extraction, purification, and quantification of the compounds are worth investing in or not must be evaluated under real aquaculture conditions.

Therefore, although significant progress has been made in the identification of antiviral agents against fish rhabdoviruses in aquaculture, new drugs and strategies to protect fish bodies should be developed in future work, which could focus on the following aspects: (i) cultivating the practical process of creating the reported antiviral compounds needs to be carried out in order to achieve a wide range of applications; (ii) extensive study on the molecular mechanisms underlying rhabdovirus-host interactions must be strengthened to obtain more knowledge on the innate antiviral immune responses in fish and the pharmacological mechanisms of different antiviral compounds; and (iii) defining the specific groups or structures of the compounds that have antiviral effects is highly necessary, as it is beneficial to the design and synthesis of new antiviral molecules or derivatives that act against fish rhabdoviruses. To sum up, future studies that engage with the development of the above topics could aid in the understanding of the antiviral mechanisms of antiviral agents and yield new antiviral molecules or strategies against fish rhabdoviruses.

## 4. Conclusions

This review demonstrated that a large number of agents exist that have the potential to fight against different strains of fish rhabdoviruses in aquaculture, and their antiviral mechanisms against virus infection were analyzed in vitro and/or in vivo. These organic compounds have unique advantages in terms of antiviral activities, although comprehensive studies need to be carried out in the future, on the premise of being able to effectively inhibit the infection and transmission of fish rhabdoviruses in aquaculture.

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