ANTIVIRAL ACTIVITY OF PHYTOBJECTS

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Abstract. Aquaculture offers a promising source of economic and healthy protein for human consumption, which can improve wellbeing. Viral diseases are the most serious type of diseases affecting aquatic animals and a major obstacle to the development of the aquaculture industry. In the background of antibiotic-free farming, the development and application of antibiotic alternatives has become one of the most important issues in aquaculture. In recent years, many medicinal plants and their active pharmaceutical ingredients have been found to be effective in the treatment and prevention of viral diseases in aquatic animals. Compared with chemical drugs and antibiotics, medicinal plants have fewer side-effects, produce little drug resistance, and exhibit low toxicity to the water environment. Most medicinal plants can effectively improve the growth performance of aquatic animals; thus, they are becoming increasingly valued and widely used in aquaculture.

Keywords: aquaculture, medicinal plants, pharmaceutical ingredients, chemical drugs, antiviral activities.

Introduction. With the increasing demand for high-quality food in modern society, aquatic products account for an increasing proportion of meat consumption [1], because modern nutrition believes that excessive intake of red meat increases the risk of rectal cancer, advocating its replacement with other foods [2], while fish and shrimp foods are also able to provide the body with essential amino acids, lipids, and minerals, representing an excellent substitute [3]. With the increasing tension in global aquatic wild fishing resources and the increasing demand for aquaculture production, the aquaculture industry has been developing rapidly while facing increased challenges, especially the steep increase in the outbreak of aquatic animal diseases due to intensive farming, resulting in the death of a large number of farmed species and serious economic losses [4, 5]. More importantly, antibiotics cannot control viral diseases; when aquatic animals are infected with viral diseases, the use of antibiotics by unprofessional farmers not only fails to alleviate the condition, but also exacerbates water pollution, resulting in an increase in mortality instead of a decrease [6].

Studies have shown that only 20–30% of antibiotics can be absorbed by farmed fish, with most of them entering the water environment [7]. The addition of antibiotics to fish and shellfish production sites via feed is a direct route to aquatic environmental

pollution [8]. Intensive aquaculture leads to the overfeeding of aquatic animals and the flow of large amounts of antibiotics from ponds to lakes and then to the oceans, contributing to the deterioration of global biodiversity [9]. Abuse of antibiotics can easily cause irreversible consequences in the ecosystem. More seriously, the harmful components of antibiotic and chemical agents will remain in aquatic products, and then enter the human body through ingestion, which can directly endanger human health, causing allergic reactions, disrupting the balance of the human microbiota, affecting the growth and development of children, and even causing cancer [10]. For example, tetracycline antibiotics can inhibit bone marrow hematopoietic function, causing human aplastic anemia [11]; furan antibiotics can cause human hemolytic anemia and acute liver necrosis [12].

In order to ensure the green and sustainable development of aquaculture, it is urgent to find alternatives to antibiotics and chemical drugs. In fact, medicinal plants are very suitable candidates for antibiotic alternatives. Medicinal plants contain a variety of active ingredients, including polysaccharides, alkaloids, organic acids, flavonoids, and phenols, which are antibacterial and antiviral, in addition to promoting the body's immune function and improving the body's ability to resist diseases [13]. In recent years, because medicinal plants have the advantages of low toxicity, few side-effects, no drug resistance, few drug residues, and low prices, they have attracted much attention in the prevention and treatment of aquatic animal diseases, and substantial progress has been made in research. For example, methanolic extracts of Urtica dioica and Pleurotus ostreatus were able to exert antibacterial effects against Aeromonas hydrophila in rainbow trout (Oncorhynchus mykiss) [14]; Zingiber officinale Roscoe enhanced disease resistance of Lates calcarifer (Bloch) against Vibrio harveyi and enhanced the nonspecific immunity of Lates calcarifer (Bloch) [15]; the compound pentagalloylglucose extracted from Galla chinensis was highly resistant to Ichthyophthirius multifiliis (Ich) and significantly increased the survival of infected channel catfish (Ictalurus punctatus) [16]; Ophiopogon japonicus extract inhibited the proliferation of white spot syndrome virus (WSSV) in Chinese mitten crab (Eriocheir sinensis) and enhanced the immune response of Chinese mitten crab [17].

Viral disease is one of the biggest obstacles facing the aquaculture industry; viruses are highly contagious and fast-spreading, have a wide host range and high mortality rate, and are the most serious type of disease affecting aquaculture species [18]. At present, most viral diseases in aquaculture are not treatable by drugs and are usually preventative in nature. Most farmers can only prevent and control viral diseases via an improvement the aquaculture environment and disinfection, but they do not achieve good results [19]. It is a very urgent task to find more effective methods of virus diseases prevention and

control, and herbal medicines have been confirmed by many studies in this regard. Therefore, this article summarizes several common aquatic viruses that are very destructive, collates the components of medicinal plants or natural compounds of plants that have antiviral effects against these viruses, and analyzes the current obstacles and problems that need to be solved in applying medicinal plants to aquaculture, aiming to provide new ideas for the prevention and treatment of viral diseases in aquatic animals.

The aim of the study. To summarize the promising antiviral properties of medicinal plants and their active pharmaceutical ingredients against aquatic viruses. Examine possible mechanisms of action and possible implications for the prevention or treatment of viral diseases in aquaculture.

Materials and Methods. A literature review was conducted including experimental data on medicinal plants and active pharmaceutical ingredients that have antiviral activity.

Results and Discussion. Infectious pancreatic necrosis virus (IPNV) belongs to the family Dictyostelium, a genus of aquatic Dictyostelium RNA viruses with icosahedral symmetry, a diameter size of about 60 nm, no capsule membrane, high pathogenicity, and high infectivity. The virus is extremely resistant to the external environment, stable to heat and acid, and insensitive to liposolvents. IPNV mainly infects fry and juveniles up to 20 weeks of age, with a very high mortality rate. Fish diseases caused by IPNV have been observed in Asia, America, and Europe, involving dozens of economic fish and shellfish species. The most obvious pathological feature is pancreatic necrosis with abnormalities in the pancreatic vesicles, islets, and almost all cells, most of which are necrotic [20]. IPNV can be spread both horizontally and vertically; fry infected with IPNV can carry the virus for several years, be excreted in semen, eggs, and feces, and subsequently infect other fish. Species of the genus *Heliotropiu* were capable of inhibiting the replication of viruses such as *Herpes simplex virus* types 1 and 2, Junin, and *Respiratory syncytial virus*.

VHSV is a member of the genus Novirhabdovirus, a family of elasmobranch viruses with a diameter of 65-80 nm, a capsule membrane, and a negative-sense single-stranded RNA genome. The virus was mainly endemic in Europe and North America, but later spread to East Asia [21]. VHSV can be excreted by sick fish and viral feces, eggs, semen, etc. and transmitted through water; it is very stable at low temperatures and can survive longer in freshwater than in seawater. *Celosia cristata* and *Raphanus sativus* are herbs frequently used in traditional medicine. Park et al. [22] tested the antiviral activity of a mixed extract of *Celosia cristata* and *Raphanus sativus* against VHSV, and they found that, if the extract was added before the EPC cells were inoculated with the virus, it had a good effect in reducing the virus titer.

Licorice (*Glycyrrhiza uralensis*, GUF) is a very common medicinal plant, and many important research projects have identified the many beneficial effects of this medicinal herb, including antimicrobial, antiviral, anti-inflammatory, antioxidant, antiprotozoal, hepatoprotective, and neuroprotective activities. Licorice contains important bioactive components, such as glycyrrhizin (GL), glycyrrhizinic acid (GLA), licorice acid, formononetin, and liquiritin [23]. Licorice extracts showed significant antiviral activity against VHSV, significantly reducing virus-induced CPE [24]. The active pharmaceutical ingredients GL and GLA also showed antiviral activity against VHSV.

The antiviral effects of curcumin have been extensively studied, and the mechanism of action mainly involves direct interference with the viral replication machinery, as well as inhibition of cellular signaling pathways essential for viral replication.

Nervous necrosis virus (NNV) is a widespread and serious infectious agent with a spherical shape, non-capsule membrane, diameter of approximately 30-40 nm, and a single positive-stranded, 2-segmented RNA molecule genome. *Gymnema sylvestre* is mainly found in tropical and subtropical regions and has unique therapeutic properties for diabetes [25]. The gymnemagenol ($C_{30}H_{50}O_4$) extracted from the leaves of *Gymnema sylvestre* had antiviral potential [26].

he grouper iridovirus (SGIV) is a novel marine fish DNA virus that belongs to the family Iridoviridae, genus Ranavirus. SGIV is a class of plasma type linear double-stranded DNA viruses with a capsule membrane, virions in the form of regular icosahedrons, and a diameter of 154-176 nm. It is highly contagious; grouper fry are susceptible to this disease, with a large number of deaths in a short period of time after the disease. SGIV is highly pathogenic, and high morbidity and mortality rates in aquatic animals have been caused by iridovirus infections worldwide in recent years [27]. *Viola philippica* is a perennial herb distributed throughout East Asia, containing anthocyanins, flavonoids, coumarins, alkaloids, phytosterol, sulfonated carbohydrate polymer, cyclotides [28]. The aqueous extracts of *V. philippica* also showed strong inhibition of SGIV infection both in vitro and in vivo [29]. RT-qPCR results showed that *V. philippica* did not damage SGIV particles, but it could interfere with SGIV binding, entry, and replication in host cells. *V. philippica* was found to have the best inhibitory effect on SGIV; it was very effective at the stage of virus binding and replication. In conclusion, the results indicate that the aqueous extract of *V. philippica* at appropriate concentrations has a strong anti-SGIV effect. Hesperidin, an important constituent of citrus fruits, has a wide range of pharmacological effects, including antiviral, anti-inflammatory, antioxidant, antitumor, and radioprotective activities [30]. The addition of 50-150 $mg \cdot kg^{-1}$ hesperidin to diets fed to Procambarus clarkii not only reduced the percentage of mortality following

WSSV infection in *P. clarkii*, but also improved their nonspecific immunity, antioxidant capacity, and growth performance [31].

The rise of aquaculture is considered to be one of the most profound changes in global food production in a century and one of the most environmentally friendly and sustainable food industries to meet the needs of humans today. However, almost all kinds of farmed aquatic animals are threatened by infections from viruses, bacteria, parasites, or other nascent and regenerating pathogenic microorganisms. Epidemics have become a constraint to the sustainable development of the aquaculture industry, especially viral diseases, which are highly contagious, spread rapidly, and have a wide range of hosts with high mortality rates. The occurrence of aquatic animal viral diseases not only leads to a decline in the quality of aquatic products and food safety hazards, but also seriously hinders the sustainable development of fisheries.

Medicinal plants and active pharmaceutical ingredients have unique advantages in terms of antiviral activity. On the premise of being able to effectively inhibit viral infection, medicinal plants and active pharmaceutical ingredients possess the advantages of low drug resistance, fewer toxic side-effects, fewer drug residues, and less pollution of the farmed water environment. Consequently, the search for new antiviral drugs with high efficiency and low toxicity from natural plants is an important way to develop antiviral drugs. At present, the development and the research of safe and efficient new herbal medicine products are getting more and more attention. An investigation found that healthy aquaculture has certain advantages and great potential for the future. The antivirus mechanisms of medicinal plants are diverse, such as direct inactivation of viral particles, interdiction of viral attachment and penetration phases, inhibition of virus replication, involvement in transcriptional regulation, disruption of virus protein synthesis or expression, inhibition of viral cell-to-cell transmission, and immunomodulatory roles. The same medicinal plants may exert different mechanisms of action against different viruses, and different medicinal plants exert even more different effects, which makes the utilization of medicinal plants promising.

Medicinal plants contain numerous active ingredients such as phenolic substances, flavonoids, alkaloids, terpenoids, pigments, starch, steroids, and essential oils. In aquaculture, medicinal plants and active ingredients can be used as growth promoters, immunostimulants, antibacterial agents, antifungal agents, antistress agents, appetite stimulants, and even aphrodisiacs, in addition to being antiviral agents. In many cases, medicinal plants exert antiviral effects by acting as immunostimulants, with antiviral activity being only an added effect. At this point, although researchers have used particular viruses to confirm whether a medicinal plant exerts an antiviral effect in fish or shellfish,

it is likely that the resistance effect is not limited to that virus. When host nonspecific immunity is increased, it is usually elevated against a wide range of pathogens. Preventing diseases by improving the immunity of fish and shellfish represents a new direction in the development of pollution-free aquaculture, which is important for disease control, health safety, and environmental protection in aquaculture.

Conclusions. Medicinal plants have great potential in terms of antivirals; whereas most of the current studies on the antiviral effects of medicinal plants focused on the inhibitory effects of plants on viruses, relatively few established a clear understanding of the antiviral mechanisms of the active pharmaceutical ingredients. In addition, few studies on the synergistic and antagonistic effects of different medicinal plants have been reported. Future research work should focus on the basic research of medicinal plants to clarify their active pharmaceutical ingredients, as well as their pharmacological and toxicological effects; accordingly, they can then be better applied in the prevention and treatment of aquatic animal diseases.

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