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A review on plants and herbal extracts against viral diseases in aquaculture

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Abstract
Aquaculture is one of the important sectors contributing significantly in the world economy. Now a day's the virus outbreak acts as an important limiting factor for aquaculture. The infection of viral disease leads to great economic loss for aquaculture industries. There are several problems associated with the use of aquaculture chemicals which include lack of knowledge for application of chemicals and antibiotics and their residual effects. In general chemical drugs are hard and time consuming task, which requires a lot of man power and financial support. Medicinal plants are now recognized to have great potential for preparing clinically useful drugs to effectively prevent and control many viruses of aquaculture origin. The present paper overviews the importance of herbal plant extracts on fish and shrimp viral diseases treatment in aquaculture. The use of plant extracts as antiviral agents in aquaculture production may also be of eco-friendly as they are biodegradable.

Keywords: Aquaculture, viral disease, herbal drugs, anti-viral activity.

1. Introduction
Aquaculture is one of the important sectors contributing significantly in the Indian economy. Fish culturists are encouraged towards intensification of culture system to increase production and profit. In such practice of fish and shrimp farming, disease becomes major threats. Disease is one of the most important constraints of fish production both in culture system, as well as wild condition. Fish production is decreased due to the occurrence of disease caused by different pathogen in aquaculture. Viral diseases have posed significant problems in aquaculture for many years. In commercial aquaculture, antibiotics were used for prevention and control the diseases, and hormones were used for growth performance but the cost of antibiotics and hormones are expensive. Several studies have been carried out to find the new compounds from plant sources at cheap and best to prevent the disease causing organisms in aquaculture. Numbers of plants have been reported as drugs against diseases in aquaculture for centuries. Herbal medicines include herbs, herbal material, herbal preparations, finished herbal products that contains parts of plant as active ingredients. Medicinal herbs are significant sources of synthetic and herbal drugs. Practically all medicinal herbs have active ingredients which are responsible for various biological activities [2]. A number of plants from both terrestrial and marine origin have already been tested against viral diseases to judge its immunostimulant efficacy [22]. There are several medicinal plants used in shrimp aquaculture which includes *Aegle marmelos, Allium sativum, Aristolochia indica, Azadirachta indica, Cassia fistula, Catharanthus roseus, Curcuma longa, Cynodon dactylon, Lantana camara, Melia azedarach, Mimosa pudica, Momordica charantia, Morus alba, Ocimum americanum, Phyllanthus amarus, Phyllanthus emblica, Psidium guajava, Solanum nigrum, Tridax procumban and Tylophora indica* [4]. Similarly, in fish aquaculture industries various herb extracts act as antiviral activity such as *Cassia alata, Calophyllum inophyllum, Clinacanthus nutans, Clinacanthus* sp., *Glinus oppositifolius, Hura crepitan, Momordica charantia, Ocimum sanctum* (red), *Ocimum sanctum* (white), *Ochrocarpus siamensis, Phyllanthus acidus, Phyllanthus amarus, Phyllanthus debelis, Phyllanthus reticulatus, Phyllanthus urinaria, Psidium guajava, Tinospora crispa, Tinaspora cordifolia* [14] The aim of this paper is to review current research work being carried out on the plants and herbal extracts against viral disease in aquaculture.

2. Viral diseases in aquaculture
Diseases and pathogens are part of every intensive aquaculture production systems. Natural mortality of 10-25% is considered to be normal in grow-out aquaculture systems. Marine

Finfish larvae (such as sea bream, seabass and yellowtail kingfish etc.) may have 5-15% of survival rate in intensive hatcheries. Normally low survival rates occurs due to combined factors such as, environmental conditions, non-specific pathogens, larvae susceptibility and low immune system development. Viruses are microscopic organisms which can infect all living cells in animal depending on specificity. Viruses are which can only replicate when an organism reproduces the virulent RNA or DNA.

Viral diseases caused large scale mortality in aquaculture and are very difficult to treat directly. During the past decade, several outbreaks of diseases devastated the aquaculture industry across the world. In marine environment, virus infections have been found in almost all organisms. The significant viral pathogen of finfish can cause serious harm to aquaculture. These includes Infectious hematopoietic necrosis virus (IHNV), Infectious pancreatic necrosis virus (IPNV) (which infect salmon), Hirame rhabdovirus (HIRRV) (flounder), the Yellowtail ascites virus (YAV) (yellow tail), Stripped jack (Sima-Aji) nervous necrosis virus (SJNNV) (mainly stripped jack) and an iridovirus (sea bream) (Maeda, 2004). Recently, the Chilean salmon industry suffered a distressed outbreak of infectious salmon anaemia (ISA). The global shrimp industry suffered major outbreaks in many countries due to poor management, as well as, uncontrolled use of antibiotics resulting in resistance developed by pathogens. Several viral diseases had serial implication for the shrimp aquaculture industry. Similarly, the culture of penaeid shrimps includes *Litopenaeus vannamei, L. stylirostris, Penaeus monodon, Penaeus japonicus,* and *Penaeus indicus*. As a minimum four virus caused pandemics have adversely affected the global penaeid shrimp farming industry since 1980. These viruses in the approximate order of their discovery are Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), and White Spot Syndrome Virus (WSSV). The socio-economic impacts of the diseases caused by these viruses have been so severe in some shrimp producing countries of throughout the world.

In India, Marine Product Export Development Authority (MPEDA) has instructed the hatcheries operators and farmers do not to use antibiotics namely chloramphenicol, nitrofurans and all their derivatives, as well as many other antibiotic groups. In 2005 FAO, have been published 'The responsible use of antibiotics in aquaculture' to increase awareness of antibiotic resistance problem in fish farming sectors. Although, different medicinal plants and herbs and/or their combinations known to contains properties includes anti-viral, anti-bacteria, anti-fungal, physiological systems supporting, hormonal balancing and many other properties.

Plant and herbs with antiviral effects in aquaculture

Botanical name	Family	Distribution	Useful parts	Biological effects in aquaculture	Reference
Oenothera biennis	Ongraceae	Japan. Eastern, northern America, U.K	Seeds, flowers and root	Antiviral and antibacterial	Shangliang *et al,* 1990
Solanum trilobatum	Solanaceae	India	Whole plant	Antibacterial and immunostimulant	Citarasu *et al*., 2003b
Stellaria aquatic	Caryophyllaceae	Japan	Whole plant	Antiviral and antibacterial	Shangliang *et al*, 1990
Acorus calamus	Aroideae	India, Burma	Rhizome	Antibacterial and immunostimul	Magdellin, 2005; Minomol, 2005; Praseetha, 2005
Cassia alata	Caesalpiniaceae	Tropics	Leaves	antiviral	Direkbusarakom, 2004
Calophyllum inophyllum	Guttiferarae	Sea coast of India	Bark, leaves and seed	antiviral	Direkbusarakom, 2004
Tinospora crispa	Menispermaceae	Tropical Subtropical India	Root and leaves	antiviral	Direkbusarakom, 2004
Momordica	Cucurbitaceae	India	Fruits, seeds and leaves	Antiviral	Direkbusarakom, 2004
Phyllanthus niruri	Euphorbiaceae	India, Sri Lanka	Whole plant	antiviral	Rani, 1999; Direkbusarakom, 2004; Immanual *et al*., 2004b
Phyllanthus urinaria	Euphorbiaceae	India, USA	Whole plant	antiviral	Direkbusarakom, 2004
Psidium guajava	Myrtaceaa	India, Bengal	Bark, fruits and leaves	antiviral	Direkbusarakom, 2004
Ocimum	Labiatae	India	Whole plant	Antiviral and antibacterial	Direkbusarakom, 2004; Citarasu *et al*., 2001
Tephrosia purpurea	Papilionaceae	Southern India	Leaves and root	Antiviral and antibacterial	Direkbusarakom, 2004; Rani, 1999
Tephrosia cordifolia	Menispermaceae	Southern India	Leaves and stem	Antiviral and Immunostimulants	Direkbusarakom, 2004; Jinish, 2002

Source: *Kolkovski, et al.,* 2011

3. Antiviral herbal medicine in aquaculture

Many herbs and plants have been used for millennia as home remedies in many cultures around the world for both human and animals. Some of these remedies have potent anti-viral, as well as, anti-bacterial and anti-fungal properties. Among them, a few have been investigated for their ability against shrimp viruses [16]. The antimicrobial activity of five Chinese herb extracts against thirteen bacterial and two viral fish pathogens have been reported by Shangliang *et al*. (1990) [41]. Biswas *et al*., 2002 reported that neem seed oil has been shown to exert to antiviral activity. Rohani *et al*., (2006) [40] has been announced that *Zataria multiflora* is an appropriate alternative source of Malachite green. The herbal activity compounds may inhibit or block the transcription of the virus to reduce the replication in the host cell and enhance the non-specific immunity. They act as immunostimulants to the host immune

system. However, the first antiviral drug (Idoxuridine) has been reported in 1950 and its first clinical use in 1962 (Bauer 1985) [7]. After this, several researchers moved toward many antiviral agents [24]. Baba *et al*., (1988) [3] proved that sulfated polysaccharides present in the algal extract could inhibit both DNA and RNA viruses.

Several plant products found to have strong antiviral activity against fish and shrimp viruses. Antiviral activities of phytochemicals have been tested against mammalian rhabdovirus - vesicular stomatitis virus (VSV) [20, 21, 9]. Guava (*Psidium guajava*) extract and ethanol extract of *Psidium guajava* was tested for anti-viral activity against the fish pathogenic viruses, infectious haematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), and Oncorhynchus masou virus (OMV) using plaque reduction in CHSE214 cell lines [16]. The direct antiviral activity of all the herbs showed antiviral activity against IHNV and OMV reducing plaques by 65-100% and 21-100%, respectively. The effect of herb extracts on virus adsorption was determined by using CHSE-214 cells pre· treated with the extracts of C. *alata, P. acidus, P. amarus* and *P. guajava* showed 100% plaque reduction for IHNV, and C. *alata* and *P. acidus* showed 92-100% plaque reduction for IPNV. Effect of the herb extracts on viral replication in the infected cell line, the extracts of O. *siamensis* and *P. acidus* showed 100% plaque reduction for OMV and IPNV [16]. Ole has been shown to possess a potent antiviral activity against other DNA and RNA viruses (Fredrickson, 2000; Ma *et al*., 2001). A commercial plant extract derived from olive tree leaf (*Olea europaea*) and its major compound, oleuropein (Ole), successfully controlled fish viruses namely salmonid rhabdovirus, viral haemorrhagic septicaemia virus (VHSV) [34].

Dasyscyphin C (C28H40O8) extract of leaves of *Eclipta prostrata* have been shown antiviral activity against fish nodavirus, grouper nervous necrosis virus (GNNV) infected SIGE (Sahul Indian Grouper Eye) cell lines under *in vitro* conditions [26]. Extracts of gymnemagenol from leaves of *Gymnema sylvestre* have been tested against the fish nodavirus, grouper nervous necrosis virus (GNNV) in infected SIGE cell lines under *in vitro* conditions. Gymnemagenol at 20 μg mL^{-1} inhibited the proliferation of GNNV to 53% at the end of the 6^{th} days by inhibiting the proliferation of GNNV-infected SIGE cells [29]. The antiviral activity of furan-2-yl acetate ($C_6H_6O_3$) extracted from *Streptomyces* VITSDK1 spp. study was carried out in cultured SIGE cells infected with fish nodavirus (FNV). These results of an immunofluorescent assay revealed a strong association between the viral capsid protein inhibition and a decline in viral replication [43].

Many herbs have been used as home remedies and some of these have potential viral properties. Several plant products found to have effective antiviral activity against Yellow Head Virus (YHV) compared to only 25 % in control group of shrimp. Anti-viral tests against the shrimp pathogenic virus, yellowhead virus (YHV), were also carried out using the injection method by Direkbusarakom *et al*. (1997) [19]. The extract of *Clinacanthus nutans* against Yellow Head Virus (YHV) of shrimp and the results indicating that this plant could effectively control YHV infection in shrimp [18]. Fifteen plants showed antiviral property against 3 viral pathogens while 8 species of plants against 4 viral pathogens. *Phyllanthus amarus* and *P. urinaria* are found to control as high as 6 viral pathogens. Many species of Phyllanthus have been widely used as herbal medicines [44]. Direkbusarakom (2004) [14] reported that *P. amarus* has very effective activity against the fish viruses such as INHV and OMV and shrimp virus YHV.

Aqueous extracts of *Cynodon dactylon* (terrestrial plant) and *Ceriops tagal* (mangrove) exhibited protective effects against WSSV in *Penaeus monodon* [4, 42]. These aqueous extract of *Cynodon dactylon* exhibited that strongest antiviral activity against WSSV at the concentration of 100 mg/kg of animal body [4]. The antiviral activity of plant extract of *C. dactylon* was studied against white spot syndrome virus (WSSV) in black tiger shrimp by in vivo testing after administration through oral route. Balasubramanian *et al*. (2008) [5] reported that the plant extract of *C. dactylon* was found to be highly effective in preventing WSSV infection with no mortality and no signs of WSD (White spot disease) at 2% and 40% mortality at 1% *in P. monodom*, respectively. Methanol extract of *M. charantia* exhibited significant antiviral against WSSV at the concentration of 150 mg/kg of animal body weight. The ethanol extract of *P. amarus* and *P. guajava* has been found to have antiviral activity against yellow head baculovirus in *P. monodon* [15]. Direkbusarakom *et al*. (1996) [16] has investigated that shrimp fed ethanol extract of *Clinacanthus nutans* had 95% survival rates when exposed to Yellow head virus (YHV) compared to only 25% survival in control group of black tiger shrimp. Direkbusarakom *et al*. (1998b) [17] has tested the extract of *Clinacanthus nutans* against yellow head virus (YHV) of shrimp and the results indicating that this plant could effectively control YHV infection in shrimp. Logambal and Michaelit (2000) [31] reported that the *Nyctanthes arbortristis* (L) have antiviral activities and its widely used plant in the traditional medicinal systems of India.

The aqueous extract of *Sargassum weighti* (seaweed) showed significant anti-WSSV property against marine shrimp, *Penaeus indicus* and freshwater crab, *Paratelphusa hydrodomous* [6]. Yogeeswaran (2007) [45] investigated that Methanolic extracts of the herbs *Acalypha indica*, *C. dactylon, P. kurroa, W. somnifera* and *Z. officinalis* effectively suppressed the WSSV after the injection with the herbal extracts and WSSV-incubated mixture. There are twenty species of Indian traditional medicinal plants such as *A. marmelos, C. dactylon, L. camara, M. charantia* and *P. amarus* exhibited strong antiviral activity against WSSV in the form of petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, methanol and ethanol extraction [4]. Achuthankutty and Desai (2004) reported the effectiveness of aqueous composite mixture of 7 Indian medicinal plants (*Aegle marmelos, Allium sativum, Curcuma longa, Cynodon dactylon, Lantana camara, Mimosa pudica* and *Ocimum sanctum*) in controlling WSSV at the rate of 15 ppm. Harikrishnan *et al*. (2010) [26] reported that intraperitoneal administration of the leaf extracts of *Punica granatum* at 50 or 100 mg kg^{-1} dose enhanced the innate immune responses and disease resistance after 8 weeks in *P. olivaceus* against natural LDV infection.

Many scientific studies have shown that allicin can actively kill a wide range of pathogens like fungi, bacteria, and even viruses [36]. Immunostimulants can be used for prophylaxis and control of WSSV includes improvement of environmental conditions, stocking of specific pathogen free (SPF) shrimp post-larvae and enhancement of disease resistance. Citarasu *et al*. (2006) [12] reported that immunostimulants are substances, which increase the non-specific defense mechanism and provide resistance against pathogenic organisms. The aqueous extract of *L. camara* and *P. amarus* and methanol extract of *A. marmelos* exhibited partial antiviral activity at the concentration of 150 mg/kg of animal body weight [4]. The extract of *Phyllanthus amarus* is a lignan composed of the compounds: niranthin, phyllanthin, and hypophyllanthin and the virucidal activities of these three substances were tested by

mixing them with WSSV, followed by injection into healthy shrimp (Loan *et al*., 2009). Gomez *et al.* (2009) [23] reported the combination of herbal extracts and probiotics works as better antiviral activity and decrease the prevalence of WSSV in *Litopenaeus vannamei*. Many plant derived compounds have been found to have non-specific immuno-stimulating effects in fish and shrimp [11, 12].

Experiment carried out by Citarasu *et al*. (2006) [12] to detect the antiviral property of 5 medicinal plants such as *C. dactylon, Aeglemarnclos, Tinospora cordifolia, Picrorhiza kurroa* and *Eclipta alba* mixed together in equal proportion of methanolic extract supplemented shrimp diet at different concentrations showed 74% survival of *P. monodon* at the rate of 800 mg/kg bodyweight. In a similar study, fed on the ethanolic leaf extract of the plant *Pongamia pinnata* the 40% percentage survival of shrimp was obtained at administering at 200 mg/kg of body weight, while 80% on administering at 300 mg/kg of body weight per day [38]. Interestingly could obtain 100% survival of *P. moodon* by fed on 2% aqueous extract of *C. dactylon* coated diet and the survived animals were PCR negative [5].

Recently in aquaculture, scores of plant extracts have been tested and used with good results in the control of viral diseases as well as bacterial diseases [2]. The anti-WSSV activity of the drug, TP22C - derived from the terrestrial plant in the host, *Litopenaeus vannamei* was evaluated by Ghosh and Chakraborty (2013) [22]. The phytochemical screening, biochemical constituents assay, elemental analysis, antioxidant property evaluation and phytochemical fingerprint profiling predicts that TP22C can protect *L. vannamei* from the WSSV infection, at a dose far below the toxicity level [22]. Guo *et al.* (2014) [25] reported the *Scutellaria baicalensis* root serves as a medicine and owns such effects as anti-virus.

4. Conclusion

Now a day's there are number of aquaculture production methods which protect the healthy environment. At the same time, number of chemicals and drugs used in aquaculture cause severe environmental problems. Recently most of the antibiotics and drugs are banned because of their negative impact and effluent remittance in the fish muscle which may cause side effect to the consumer. Herbal drugs present a viable alternative to antibiotics and other banned drugs being safer for the cultured organism as well as, the environment. Most of the herbs and herbal extracts can be given orally, which is the most convenient method of antiviral enhancement. Here the problem is farmer does not know about the importance of herbal drugs. So it is necessary to organize the awareness programme on biological and eco-friendly approach of herbal medicines usage in aquaculture to the farmers. It will reduce the production risk, negative impact and production cost and also increasing fish production with sustainable way.

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