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Review on the antiviral activity of natural compounds against Tilapia Lake virus

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Abstract

Aquaculture has become the fastest growing food sector providing more than half of the fish supply chain. Tilapia is an important finfish aquaculture species because of its affordability, high-quality protein and nutrients, tolerance to high-density aquaculture, and reputation for disease resistance, tilapia farming has become a significant part of aquaculture. There was severe challenge occurred due to disease outbreaks, high stocking density and overuse of antibiotics. Tilapia was infected with several bacterial and viral infection, among them TiLV show severe loss to tilapia farming. Tilapia lake virus (TiLV) is a new virus that has had a significant impact on the global tilapia farming. Compared with chemical drugs and antibiotics, natural medicines have lesser side-effects, produce drug resistance, and exhibit low toxicity to the water environment. Most of the medicinal compounds can effectively improve the growth performance and disease resistance for aquatic animals. The present review summarizes the antiviral activities of natural compounds and their importance against fish virus. Furthermore, usage of homeopathy and herbal medicines will have positive impact on the prevention or treatment of viral diseases in aquaculture. This article will have clear explanation about herbal and homeopathy medicines which will combat viral disease in Aquaculture sector.

Keywords: Tilapia fishes, tilapia lake virus, fish cell line, natural compounds, antiviral property

Introduction

Aquaculture is indeed playing a significant role in increasing global fish production and satisfying expanding demand for fisheries products, and it will continue to do so in the future. Capture fisheries production has stopped growing, and it is no longer thought to be capable of meeting the expanding worldwide demand for fisheries products. Aquaculture output has been continuously increasing in recent years, and it is now the fastest growing food production industry in the world. It has become an important part of overall development initiatives in many parts of the globe. Aquaculture is one of the most fast-growing segments of the global livestock industry. India, Bangladesh, Egypt, Myanmar, and Brazil concentrate mostly on inland finfish aquaculture, while their potential for mariculture finfish output remains virtually unexplored.

Tilapia culture

Tilapia is the common name for a group of cichlid fish that live in freshwater streams, ponds, rivers, and lakes, as well as brackish water. Tilapias, once considered an invasive species, are increasingly becoming more important in aquaculture. The tilapia is the world's second-most-important finfish aquaculture species. Because of its affordability, high-quality protein and nutrients, tolerance to high-density aquaculture, and reputation for disease resistance, tilapia farming has become a significant part of aquaculture. China remains the single largest producer of freshwater fish for export and domestic consumption for 56% of the global output in 2017.

Tilapia production accounts for about 40% of fisheries sector in aquaculture. There are 1524 different species of tilapia. Their native lands range from southern Africa and Madagascar to northern Syria. Three genera are responsible for economically important species such as table fish (Tilapia, Oreochromis, and Sarotherodon). Aside from their nutritional value, certain species are beneficial for managing aquatic vegetation. They described 77 tilapia species (along with a few subspecies), while Jhingran *et al.* (1973) ^[37] included 22 species

that have been used in experimental or industrial scale fish cultivation. *Oreochromis niloticus* (Nile tilapia), *Oreochromis mossambicus* (Mozambique tilapia), *Oreochromis aureus* (Blue tilapia), *Oreochromis hornorum* (Zanzibar tilapia), and *Oreochromis* hybrids are the most widely cultivated tilapia species (Red tilapia).

Oreochromis niloticus, sometimes known as the Nile tilapia, is one of the most significant fish species in tropical and sub-tropical aquaculture. It is a significant source of animal protein and profit all around the world. Asian countries are the largest producers and consumers of tilapia. China is the leading producer of *Oreochromis niloticus* alone, accounting for approximately one-third of global production. Nile tilapia account for more than 90% of all commercially farmed tilapia outside of Africa. Because of its resemblance to the marine red snapper, which gives it a high market value, the hybrid "red" tilapia has become increasingly popular for aquaculture. Genetic mutations were used to create the first red tilapias. In China, Bangladesh, Indonesia, Laos, Malaysia, Taiwan, and the Philippines, tilapia aquaculture has grown in popularity. The global tilapia production at almost 7 million tonnes in 2020. Many countries culture this species, the countries like China, Thailand, Ecuador, Egypt, and Indonesia are the top producers, while the United States is the largest importer Harvey *et al.* (2015) [31]. There was no report of any viral disease in tilapia until 2009, during summer there will be mass mortality observed in 2009 from different parts of Israel. The annual global production of farmed tilapia has been steadily increasing in recent years. China (1.8 million tonnes), Indonesia (1.1 million tonnes), and Egypt (875 thousand tonnes) were the top three tilapia producers. Bangladesh, Vietnam, the Philippines, Brazil, and Thailand are among the top tilapia producers in the world Cai *et al.* (2017) [16].

Despite all of the apparent advantages of tilapia farming, they have succumbed to different diseases, as have all highly grown fish. Many cases of bacterial infections in tilapia have been reported, including infections caused by *Flavobacterium columnare*, *Aeromonas* species, and *Streptococcus* spp. *Aeromonads* like *A. hydrophila* and *A. veronii* spread horizontally in fish, causing haemorrhagic sickness, ulcerative syndrome, and Motile *Aeromonas* *Septicaemia*, with significant mortality rates, especially in farmed warm water fish. A case of spontaneous TiLV and *A. veronii* co-infection in Malaysian red hybrid tilapia was recently reported Amal *et al.* (2018) [5]. *Aeromonas motile mesophilic* is zoonotic, meaning it may spread between animals and people, causing a wide range of infectious diseases in a wide range of hosts, including amphibians, birds, fish, reptiles, and mammals. A variety of viruses have been linked to disease in farmed fish, but only a few have been linked to tilapia. There will be reporting of eight viral diseases in tilapia which comprise of three RNA and five DNA viruses. The reported RNA viruses are *Tilapia Lake virus* (TiLV), *Nervous necrosis virus* (NNV) and *Infectious pancreatic necrosis virus* (IPNV) while the five reported DNA viruses include the *Bohle iridovirus* (BIV), *infectious spleen and kidney necrosis virus* (ISKNV), *Lymphocystivirus*, *Iridovirus-like* (IriVL) and *Tilapia larvae encephalitis virus* (TLEV) Machimbirike *et al.* (2019) [48]. Until recently, when a novel virus called tilapia lake virus emerged, none of the previously described viruses were worldwide common (TiLV). It is officially known as *Tilapia*

tilapinevirus of the genus *Tilapinevirus* and family *Amnoonviridae* here it is referred as TiLV. TiLV, NNV and ISKNV are the virus causing severe or potential threats to farmed tilapia industry in ASEAN countries Dong *et al.* (2017) [26].

Tilapia lake virus

Tilapia lake virus (TiLV) is a new virus that has had a significant impact on the global tilapia farming business and food security Jansen *et al.* (2019) [36]. Based on its shape of round to oval enveloped and filamentous/tubular viral particles with negative-sense, 10-segment RNA genomes and a total length of 10,323 kb, the virus was classed as an Orthomyxo-like virus Del-Pozo *et al.* (2017) [22]. Open reading frames (ORFs) can be found in each of the ten segments, and each ORF can code for up to 14 different proteins. With a nucleotide size of 1.641 kb, segment 1 is the largest in the TiLV genome. Segments 1, 2, 4, and 5 have a high level of homology with the Dhori virus, whereas segments 6, 7, and 8 have a significant evolutionary relationship with the influenza virus. Segment 3 has a low level of homology with the infectious salmon anaemia virus Acharya *et al.* (2019) [2]. By April 2020, TiLV had been detected in 16 countries on four continents, Following the first discovery of a novel disease in Ecuador, dubbed syncytial hepatitis of tilapia, TiLV was detected in Israel in 2014 Eyngor *et al.* (2014) [27]; Bacharach *et al.* (2016) [8]; Ferguson *et al.* (2014) [29]. TiLV outbreaks have also been documented in Peru Pulido *et al.* (2019) [72], Ecuador Subramaniam *et al.* (2019) [85], Egypt Fathi *et al.* (2017) [28]; Nicholson *et al.* (2017) [63], India Behera *et al.* (2018) [13], Thailand Dong *et al.* (2017) [26]; Surachetpong *et al.* (2017) [88], and Malaysia Abdullah *et al.* (2018) [1]; Amal *et al.* (2018) [5], Bangladesh Chaput *et al.* (2020) [17], Tanzania Mugimba *et al.* (2018) [59], Uganda Mugimba *et al.* (2018) [59], USA Ahasan *et al.* (2020) [3], Colombia Tsofack *et al.* (2017) [41]. On a worldwide scale, the virus's high mortality rates (20-90%) and quick spread have affected the livelihoods of millions of tilapia farmers Jansen *et al.* (2019) [36]. Tilapia can cause up to 90% mortality in farms of Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), and hybrid tilapia (*O. niloticus* x *O. aureus*) Dong *et al.* (2017) [26]; Eyngor *et al.* (2014) [27]; Ferguson *et al.* (2014) [29]; Ecuador Ferguson *et al.* (2014) [29], Israel Eyngor *et al.* (2014) [27], Colombia Kembou Tsofack *et al.* (2017) [41], Egypt Fathi *et al.* (2017) [28]; Nicholson *et al.* (2017) [63], and Thailand Fathi *et al.* (2017) [28]. Dong *et al.* (2017) [26]; Surachetpong *et al.* (2017) [88]. Nested RT-PCR, semi-nested RT-PCR, SYBR quantitative RT-PCR, and in situ hybridization have all been reported as TiLV detection methods Bacharach *et al.* (2016) [8]; Dong *et al.* (2017) [26]; Kembou Tsofack *et al.* (2017) [41]. TiLV infection has only been found in tilapia and hybrid species, with common carp and grey mullet grown in ponds with TiLV-infected tilapia showing no clinical indications of the disease Eyngor *et al.* (2014) [27]; Fathi *et al.* (2017) [28]. TiLV was found in two samples from ornamental African cichlids with TiLV symptoms, in addition to tilapia Kembou Tsofack *et al.* (2017) [41]. The majority of warm-water fish were TiLV-resistant, with no mortality or clinical symptoms of infection. In giant gourami (*Osphronemus goramy*) infected with TiLV, however, significant mortality, severe clinical symptoms, and histological abnormalities were detected. Furthermore, TiLV genomic RNA was only found in the

livers of giant gourami and red hybrid tilapia, but not in liver samples from other fish species, indicating that these two species are TiLV susceptible Jaemwimol *et al.* (2018) [35].

Their investigation was able to detect TiLV antigens in both experimental and naturally TiLV-infected fish using a synthetic rabbit polyclonal IgG antibody produced against the TiLV antigen. TiLV infection displays cellular tropism using immunohistochemistry Piewbang *et al.* (2021) [68]. In this work, researchers analysed at the immunological effects and protective effectiveness of formaldehyde and propiolactone-inactivated vaccinations against TiLV in tilapia with and without the Montanide IMS 1312 VG adjuvant. The propiolactone-inactivated TiLV vaccine, when combined with the adjuvant Montanide IMS 1312 VG and booster immunizations, can provide tilapia with substantial levels of resistance against virus challenge. The majority of tilapia developed antibody responses to this vaccine, and titers increased dramatically after booster immunisation. This suggests that this vaccination could be useful in preventing TiLV infection in locations where TiLV epidemics are a concern Zeng *et al.* (2021) [101]. It has a number of advantages over traditional vaccinations, including ease of preparation, stability, and a relatively low cost. DNA vaccines are currently being utilised to manage or reduce virus infection in humans, animals, and fish Corbeil *et al.*, (2000) [18]. TiLV-ORF10 was used to prepare DNA vaccines and investigate antiviral effects in this case Yu *et al.* (2021) [100]. They tested the ability of two variants of a simple water-based inactivated vaccination for TiLV (HKV and FKV) to protect tilapia against the virus in their study. In vaccinated fish, both HKV and FKV were able to confer reasonably significant levels of protection (RPS, 71.3 percent vs. 79.6 percent) Mai *et al.* (2021) [50]. They developed the most effective approach for detecting TiLV antigen and confirmed the rabbit polyclonal IgG antibody immunised with a pure TiLV antigen. A unique band at 36 kDa was found in both the vaccinated rabbit serum and the TiLV-exposed fish serum. This is the first study to show that ribavirin effectively reduces the cytopathic effect of TiLV infection in fish cells. Ribavirin also reduces TiLV replication and enhances cell survival in a dose-dependent manner, according to the researchers. Despite this, they discovered that ribavirin can cause cell toxicity in E-11 cells at high concentrations. They established the efficacy of antiviral treatment against TiLV in their experiment Lertwanakarn *et al.* (2021) [45]. This study found that HKV and FKV vaccination of tilapia broodstock results in a protective antibody response against TiLV, which can then be passed on to fertilised eggs and larvae to promote maternal immunity. For maternal antibody transmission, HKV appears to have a higher potential than FKV Mai *et al.* (2022) [49].

In their study, six immune response genes were found to be elevated three and six weeks after TiLV inoculation, they have found significant increases in the expression of cytokine genes encoding TNF-, IL-1, and IFN-, as well as the CD4 and MHC adaptive immune genes MHC-I and MHC-II in the spleens and kidneys of most of the vaccinated tilapia. The use of the adjuvant in combination with booster immunizations, in particular, resulted in an increase in immune response and protection. Cytokines are a type of cell signalling molecule that connects the innate and adaptive immune systems Zou *et al.* (2016) [102]. The use of

zebrafish as an animal model to investigate immune responses after infection with tilapia lake virus (TiLV), a new virus linked to significant morbidity and mortality in both farmed and wild tilapia populations. The death of zebrafish infected with TiLV by i.p. injection was, on the other hand, quite low, compared to tilapia mortality, which was over 80% during challenge studies in the lab. TiLV replicates in a variety of zebrafish organs, with the brain containing the most virus at 6 dpi. TiLV also activates the adaptive immune response and triggers a strong type I IFN response, which may both have a role in reducing virus replication and boosting fish survival Mojzesz *et al.* (2020) [73]. The zebrafish is rising in popularity as a model organism for studying host-pathogen interactions. The larvae provide a different model system to investigate primarily innate immunity because the adaptive immune system of zebrafish matures between 4 and 6 weeks after fertilisation Lam *et al.* (2006) [44]. Clear histopathology alterations in the brain of TiLV-infected tilapia have also been detected. Lesions, focal haemorrhages, capillary congestion in both white and grey matter, and gliosis foci Eyngor *et al.* (2014) [27]; Jansen *et al.* (2019) [36]; Tattiyapong *et al.* (2017) [90]. Despite the virus being injected intramuscularly into broodstock in this experiment, the virus was later identified in the blood, liver, and reproductive organs, as shown by PCR, ISH, and cell culture. This research demonstrates that TiLV produced systemic infection in tilapia broodstock and that the virus can circulate through the bloodstream into both male and female reproductive organs. Contaminated female fish generated infected eggs, which resulted in infected fertilised eggs when fertilised with sperm from infected male broodstock Dong *et al.* (2020) [25]. Natural outbreaks of TiLV in broodstock and fry in two tilapia hatcheries were confirmed in their research. TiLV genomic RNA was found in infected broodstock's liver and reproductive organs, and infective virus was isolated from a susceptible cell line. TiLV was found in the ovary and testis of naturally infected and experimentally challenged fish using an insitu hybridization technique. Furthermore, the presence of TiLV genomic RNA and active virus in the testis and ovary of 2-day-old fry revealed that the virus might be transferred from infected broodstock to progenies Yamkasem *et al.* (2019) [99].

In unexposed red tilapia challenged by TiLV IP-injection or cohabitation with TiLV-infected fish, the average cumulative mortality was 70% and 47%, accordingly. The IP-challenged fish showed clinical symptoms of TiLV infections within 4-5 dpc, whereas the cohabitation-challenged fish displayed clinical signs within 9-12 dpc. Survivors of past TiLV infections who were re-challenged by IP-injection or cohabitation showed no clinical symptoms or mortality. At 28 days after the main challenge, an RT-qPCR assay revealed no TiLV in the liver and spleen of the surviving fish. At 1, 3, and 7 days after being re-challenged with TiLV, there was no evidence of the virus in the liver or spleen. At 28 days after the first challenge (0 days after the re-challenge), the majority of the rechallenged fish have circulating antibodies at a particular level, and could produce fast antibody production after secondary exposure to TiLV within 7 days. Additional challenge studies indicated that individuals who had been in the lab for two months since their first exposure to TiLV had developed protective immunity against repeated exposure to

the virus Tattiyapong *et al.* (2020) ^[91]. The observation of zebrafish larvae is sensitive to tilapia lake virus and that systemic TiLV injection causes substantial mortality at 4-5 days post infection. This is in contrast to adult zebrafish, which had extremely minimal mortality with TiLV infection Widziolek *et al.* (2021) ^[96].

Cell culture isolation is the important standard for virus identification, according to the OIE. Studies on TiLV isolation utilising eight different cell lines (tilapia primary brain cells, E11, CHSE214, BF2, BB, EPC, KF1, RTG2, and FHM) revealed that only the E11 cell line and primary tilapia brain cells displayed a cytopathic effect (CPE) cell lines are not available from *O. niloticus* Eyngor *et al.* (2014) ^[27]. TiLV has been obtained from the brains of infected tilapia in a number of experiments Dong *et al.* (2017) ^[26]; Eyngor *et al.* (2014) ^[27]; Ferguson *et al.* (2014) ^[29]; Kembou Tsofack *et al.* (2017) ^[41]. According to Reed and Muench's (1938) approach, virus titres were expressed as the 50% tissue culture infective dose (TCID₅₀). Isolating viruses and understanding virus-host interactions require the use of cell lines Crane *et al.* (2011) ^[19]. Because fish viruses are often host specific, a cell line produced from a certain fish species is better suited to analyzing the viruses that have been identified from that species Pandey *et al.* (2013) ^[64]. They produced two cell lines from the liver and brain of Nile tilapia, which are the main target organs of TiLV, and designated them as OnIL and OnLB Swaminathan *et al.* (2018) ^[89]. Using the explant approach, a cell line was obtained from the heart of *O. niloticus*. Many cell lines have already been generated from heart tissue of many fish species, including SHHT (*Channa striatus*), SICH (*Catla catla*), RH (*Labeo rohita*), SPH (*Lateolabrax japonicus*), GH (*Epinephelus awoara*), PH (*Sardinops sagax*), RGH (*Epinephelus quoyanus*) and TH (*O. mossambicus*) Nanthini *et al.* (2019) ^[61]. The vulnerability of OnH cells to tilapia lake virus was investigated in this research, and the cytopathic effects in the OnH cell line comprised cell elongation and rounding, followed by death and detachment after TiLV inoculation Yadav *et al.* (2020) ^[97].

They used explant method to produce a cell line from the caudal fin of striped catfish in their experiment and the vulnerability of the PHF cell line to tilapia lake virus (TiLV) was investigated in this work. PHF cells, on the other hand, showed no CPE after 14 days and even after three blind passages. This indicated that the cell line was not TiLV permissive, and so could not be used to detect TiLV in suspicious samples Soni *et al.* (2018) ^[84]. The explant approach is employed for cell line generation because it is simple and quick, and it does not cause harm to the cell surface like the enzymatic method does Avella *et al.* (1994) ^[7]. Caudal fin was used for cell line creation because it has a high regeneration potential and more importantly, it can be removed from fish without sacrificing the fish Rathore *et al.* (2007) ^[75]. SSN1 and CFF cell lines derived from *Channa striatus* and *Pristolepis fasciatus*, respectively, have been observed to cause CPE in TiLV-infected heterologous cell lines Eyngor *et al.* (2014) ^[27]; Behera *et al.* (2018) ^[13]. Both Nile and red tilapia were injected with cell culture suspension made from TiLV infected E-11 cells in their study to see if TiLV can induce disease in native susceptible fish. Clinical symptoms such as epidermal erosion, haemorrhages, and abdominal distension were found in challenged fish at 3 to 5 dpi. These clinical indications in the experimentally challenged fish were similar to those

observed in the field Tattiyapong *et al.* (2017) ^[90]. This research indicated that Aulonocara spp. are very vulnerable to TiLV when infected via intracoelomic injection, as evidenced by ISH, RT-qPCR, and virus isolation, among other methods. After exposure to TiLV, the challenged ornamental African cichlids had a high mortality rate, as well as severe clinical symptoms and histological abnormalities Yamkasem *et al.* (2021) ^[98].

Experimental infection using tilapia lake virus

In this challenge experiment with TiLV, there will be observing of fish mortality on 5 days post challenge and cumulative mortality result in ornamental African cichlids *Aulonocara spp* with 56.25% and the viral copies was high in all the three organs in ornamental fish Yamkasem *et al.* (2021) ^[98]. In this study, they evaluated the experimental infection of three carps (*C. catla*, *C. mrigala*, and *H. molitrix*) and the result shows that these fishes are resistant to virus and non-susceptible to TiLV. There will be no clinical sign and histopathological changes were observed in this study Pradhan *et al.* (2021) ^[71]. In their research an experimental challenge with TiLV infection was observed in 10 warm water fish species consist of giant gourami (*Osphronemus goramy*), snakeskin gourami (*Trichogaster pectoralis*), iridescent shark (*Pangasianodon hypophthalmus*), walking catfish (*Clarias macrocephalus*), striped snake-head fish (*Channa striata*), climbing perch (*Anabas testudineus*), common carp (*Cyprinus carpio*), silver barb (*Barbodes gonionotus*), Asian sea bass (*Lates calcarifer*), and red hybrid tilapia (*Oreochromis spp.*) and the result revealed that red hybrid tilapia and giant gourami developed a clinical sign and mortality observed between 60-100% Jaemwimol *et al.* (2018) ^[35]. During the 12-day experimental period, rohu treated with TiLV showed no clinical symptoms and no mortality Pradhan *et al.* (2020) ^[70]. In this study the adult zebra fish was infected with TiLV by intraperitoneal injection and the infection was observed in liver, spleen and kidney. They have analysed antiviral response against TiLV using zebra fishes Rakus *et al.* (2021) ^[73]. This study shows that the infected red tilapia (66%) and Nile tilapia (86%) showed severe mortality using TiLV. The mortality was observed within 4-12 days of post infection Tattiyapong *et al.* (2017) ^[90]. Infection of TiLV in Mozambique tilapia shows mortality and observation of clinical signs Waiyemitra *et al.* (2021) ^[95].

Antiviral compounds used in aquaculture

In mammalian systems, LJ001 has proven to be a broad-spectrum antiviral against enveloped viruses. LJ001 was found to be effective against a fish virus both *in vitro* and *in vivo* in the current study. LJ001 was also non-cytotoxic to fish cells at antiviral doses. They further expect that virus inactivated by LJ001 may stimulate a protective innate immunological response in the host, which might reduce viral load and shedding, or even prevent infection entirely. IHN was used as a model for testing the broad-spectrum antiviral LJ001 for use in aquaculture Balmer *et al.* (2017) ^[11]. Fish cytokines and cytokine-induced proteins have been found to play important roles in bacterial and viral pathogen defence Zou *et al.* (2016) ^[102]. Type I interferons are main antiviral cytokines which will play an important role against viral pathogens in all vertebrates. Through recognition by different pattern recognition receptors, Type I IFNs are synthesized in response to different pathogens or pathogen-

associated molecular patterns (PAMPs) Honda *et al.* (2005) [32]. Certain experiment has been done to find the efficacy of recombinant type I IFNs using genes from different fish species (grouper, salmon, sea bass, and tilapia) to fight nervous necrosis virus (NNV) in the grouper *Epinephelus malabaricus*, the sea bass and tilapia shows IFNs exerted antiviral effects in both *in vitro* and *in vivo*. The grouper and salmon IFNs exhibited antiviral activity Kuo *et al.* (2016) [43]. The effectiveness of LJ001 and two structurally related compounds, JL122 and JL118, against IHNV, VHSV, and SVCV was compared. Among these compounds JL122 will inhibit the virus replication of selected three fish rhabdoviruses *in vitro* Balmer *et al.* (2018) [10]. They have done a study of using ethanol and methanol extract of marine macro algae in fish cell lines. The antiviral activity of marine algae was performed against *viral hemorrhagic septicemia* and *infectious pancreatic necrosis viruses* Monteiro *et al.* (2021) [58]. Coumarin plant derivative compound C2 act as an antiviral effect against SVCV infection *in vivo*. C2 will prevent horizontal transmission of infection against SVCV Liu *et al.* (2019) [46]. Their study proved the antiviral activity of 12 natural compounds and 7 were reacted as antiviral activity against SVCV. The highest inhibition of arctigenin compound results in blocking of viral replication Shen *et al.* (2018) [80]. They have used the type of homopolysaccharide, dextrans of bacterial origin have shown antiviral activity against fish viruses. Purified dextrans produced by lactic acid bacteria has been proven to be effective against salmonid viruses, IPNV, and IHNV N  cher-V  zquez *et al.* (2015) []. The antiviral activity of an ethanol extract of *Psidium gugajava* leaves was tested against *Infectious Haematopoietic Necrosis Virus* (IHNV), *Infectious Pancreatic Necrosis Virus* (IPNV), and *Oncorhynchus masou virus* (OMV) using CHSE-214 cell lines by plaque reduction assay. BOA, a new ARG derivative, was developed and synthesised to have excellent antiviral activity against aquatic rhabdoviruses in host cells. BOA did not directly alter viral infectivity or interfere with viral adsorption on a mechanical level Shen *et al.* (2020) [81]. *P. amarus* and *P. gugajava* ethanol extracts were found to have antiviral action in *P. monodon* against *yellow head baculovirus* Direkbusarakom *et al.* (1993) [23]. Only five of the 20 plant extracts studied have antiviral action against WSSV (*A. marmelos*, *C. dactylon*, *L. camara*, *M. charantia*, and *P. amarus*). *C. dactylon's* aqueous extract had the strongest antiviral activity against WSSV, while *M. charantia's* methanol extract had substantial antiviral activity. Other extracts were unable to completely inactivate WSSV Balasubramanian *et al.* (2007) [9]. The compound amino-fluorophenyl will act as an antiviral activity against *white spot syndrome virus* in *Litopenaeus vannamei* larvae and it reduces the viral load and increased the survivality against WSSV infected shrimp Shan *et al.* (2021) [79]. This study is the first to reveal that ethanolic leaves extract of the plant *P. pinnata* impregnated shrimp feed significantly reduced the mortality of WSSV-infected shrimp and these plant extract will act as an antiviral activity against WSSV Rameshthangam *et al.* (2007) [74]. The ethanolic extract of *Cynodon dactylon* on WSSV in tiger shrimp *Penaeus monodon* juveniles shows antiviral activity. It was administered via intramuscular injection and result in good survivality of shrimps Howlader *et al.* (2020) [33]. The antiviral compound Genipin (GN) is extracted from the fruit of *Gardenia jasminoides* used against crayfish *Procambarus*

clarkii and shrimp *Litopenaeus vannamei*. *In vitro* study Genipin extract shows the highest inhibition on WSSV Huang *et al.* (2019) [34]. The extracted polysaccharide from the marine green algae (*Acrosiphonia orientalis*) shows antiviral activity against the shrimp pathogen. The study was done by experimental challenge with WSSV and extract was given through feed for inhibition of virus replication Manilal *et al.* (2009) [51]. There will be development of more antiviral compounds against various fish virus for the prevention of disease in aquaculture sector for the future purpose.

Vaccines in aquaculture

Vaccines are biological related preparation establish to improve immune response for particular disease or group of disease. Fish are cold blooded animal as it will respond to vaccine based on the temperature of water. If the temperature will be higher the vaccine respond to the fish will be faster. During the past 20 years period vaccine play an important role in controlling infectious disease and cost-effective method in aquaculture. In previous years, there will be use of antibiotic for the prevention of bacterial disease and the use of antibiotic has not given proper solution for the disease outbreak. There will be a development of more problems associated with the use of antibiotics in aquaculture. The use of antibiotics results in development of antimicrobial resistance factor against bacterial diseases. For the alternative source there will use of vaccination treatment in fish and shrimp diseases. Vaccination is the method of lowering the risk of disease occurrence in aquaculture. Vaccine will be immunized by via injection through intramuscularly, intraperitoneal and oral route or the vaccine will be incorporated into the feed for disease prevention. The administration of vaccine has both advantage and disadvantage, it is approved that only the injection and immersion routes have given enough protection in commercial vaccines. The method of injection will be impressive but due to side effect and cost effective to seek for alternative approach Plant *et al.* (2011) [69]. The vaccine will provide proper immunoprotection against disease outbreak. IPNV, IHNV, SVC virus, salmon pancreas disease virus, and ISA virus vaccines are the commercially available viral vaccines for fish virus Salgado-Miranda *et al.* (2013) [77]. Intra-abdominal adhesions, inflammation, granulomatous peritonitis, and pigmentation near the injection site Midtlyng *et al.* (1996) [57], as well as spinal abnormalities, are all side effects of vaccine mixed with adjuvant Berg *et al.* (2006) [14]. Vaccine may cause severe reaction if it is wrongly administered to the fish and small size fish will not properly respond to this method due to their less immune response of smaller size fish Komar *et al.* (2004) [42]. In spite of reducing this risk, homeopathy medicine can be used against fish virus and it will aid in a safe and cost-efficient in aquaculture production. There will not be any negative impact of using these homeopathy medicine and it is eco-friendly in nature.

Homeopathy medicine

Homeopathy is a science created by Samuel Hahnemann in 1796. Technical applications of infinitesimal dilutions include: Hahnemann dilutes and then vigorously agitates his formulations in order to lessen the toxicity of the ingredients chosen using the principle of similarity. The solution is violently shaken (succussion) after each dilution, either

manually or mechanically, to maintain its pharmacological properties after significant dilutions. Despite the absence of scientific evidence, it is regarded as an alternative medicine because of its low cost, less side effects, and occasional failure of traditional treatment methods Baran *et al.* (2014) [12]. At each level of potentization, there is serial dilution and agitation (with vigorous strokes) or trituration. Trituration is the process of finely grinding a starting substance with lactose, which is utilised when the starting substance is insoluble in water or alcohol. When diluted 1:9 and 1:99, the potency levels are indicated as "x" (or "d") and "c," respectively. A dilution without agitation (or trituration) is regarded to be devoid of therapeutic efficacy and should not be referred to as potency. Potency 12cH, for example, was diluted 1:99 12 times with strong strokes at each dilution level; „H” stands for Hahnemann's technique. According to Avogadro's number, no molecule of the beginning chemical should have a potency greater than 12CH. However, considerably greater centesimal potencies, such as 200, 1,000, 10,000cH, and so on, are commonly used in homeopathy therapeutic treatment with good outcomes.

Treatment with homeopathy pharmaceuticals has been proven to be effective against bacterial infections in several studies Peckham *et al.* (2014) [67], however there are few reports that homeopathy drugs are useful against bacterial diseases. Homeopathy medicine will represent the nanomedicine and improve the immune response in the cellular level Upadhyay *et al.* (2011) [93]. Homeopathy medicines are extracted or obtained from natural or synthetic sources including minerals and chemicals, plant materials including roots, stems, leaves, lowers, bark, pollen, lichen, moss, ferns and algae, microorganisms including fungi, bacteria, viruses and plant parasites including animal organs, tissues, secretions and cell lines; human materials include tissues, secretions, hormones, and cell lines. Many antibiotics and chemicals used in aquaculture effect on increasing such as water and soil contamination, as well as the creation of unhealthy aquaculture products Doehring *et al.* (2016) [24].

Homeopathy medicine role in aquaculture

Homeopathy can be used to heal fish because it works with all living organisms. Instead, then trying to force a pill down a struggling fish's throat, remedies can be used to avoid some fish ailments. There is an easier solution, however simply medication the water in which it swims. The doses should be determined by the water area as well as the fish stocking density. For example, a small tank containing water and infected fish can be treated by dissolving one pill per litre in the water and waiting 24 hours to see if the fish respond. The sick fishes can be treated in groups for greater areas and quantities of fish. Overtreatment of the fishes by exposing them to medicated water in the sun may lead them to become ill. For appropriate homeopathy treatment, proper symptom identification is essential Doehring *et al.* (2016) [24]. During the time of gonadal differentiation, a comparison study was undertaken in which the effect of homeopathy complex was assessed against the performance, gonadal response, gills, and liver histology of Nile tilapias. The Nile tilapia (*Oreochromis niloticus*) has been revealed to be one of the most profitable fish species in modern aquaculture practises Valentim *et al.* (2008) [94]. Homeopathy will have an

important role in marine fish aquaculture production Mazón-Suástegui *et al.* (2016) [53].

Homeopathy medicines exhibited a statistically significant influence on general health, nutrition, and immunity in coccidia-infested juvenile snapper fish *L. guttatus*. Apart from increased mucin production in gill tissue and increased production of neutrophils and lymphocytes in blood, which could act as macrophages against the presence of intestinal coccidia, homeopathy treated fish showed increased mucin production in gill tissue, with associated increased production of neutrophils and lymphocytes in blood Rosero-García *et al.* (2019) [21]. The homeopathy combination lowered cortisol levels and increased performance in Nile tilapia (*O. niloticus*) under the same experimental studies as the control group Merlini *et al.* (2014) [56]. When compared to animals treated with the synthetic hormone and controls, Nile tilapia fingerlings (*Oreochromis niloticus*) fed with the homeopathy complex Homeopatila RS demonstrated stronger hypertrophy of muscle fibres, smaller average body size, and higher survival Júnior *et al.* (2012) [40]. The four-hour transportation induced physiological changes in juvenile pacu (*P. mesopotamicus*), and the homeopathy compound, when supplied in water or diet, did not reduce stress reactions in the doses and period examined. Dietary sucrose supplementation changed cortisol and blood glucose levels, showing that it has a calming effect on these stress markers and it shows better results when compared with homeopathy medicine De Oliveira Feitosa *et al.* (2013) [20]. Their research found that a homeopathy medication formulated from pathogenic bacteria at decimal and centesimal dilutions (1D, 7C) had the capacity to stimulate the scallop *Argopecten ventricosus* immunomodulatory response and antioxidant system, as well as promote growth. T3 (AcF 1D + MsS 1D) is a homeopathy medication made from sodium metasilicate and phosphoric acid mother tinctures. The centesimal homeopathy dynamization (1:100 dilution/succussion) of a commercial homeopathy medication manufactured from the venom of the blue scorpion *Rhopalurus junceus* was used in Treatment T5 (ViD 31C). The final dilution vehicle was distilled water and these T3 and T5 homeopathy complex shows increased immune modulatory response López-Carvalho *et al.* (2019) [47]. The nosodes are broad-spectrum preparations made from biologic materials such as cultures or clinical samples of microorganisms (such as bacteria, fungi, and viruses), parasites, diseased tissues (such as malignant tissues), or decomposition products from animals and humans Shah *et al.* (2014) [78]. For the first time, the composition and diversity of the gastrointestinal microbiota of the sea mollusk *A. ventricosus* has been reported, with the phyla Proteobacteria, Actinobacteria, and Bacteroidetes dominating. *Microbacterium maritropicum* (T1, T3) and *Symbiobacterium toebii* both developed and survived in the presence of (T2, T5) homeopathy treatment Mazón-Suástegui *et al.* (2019) [55]. The impact of the homeopathy drug *Natrum muriaticum* 30 c on *Puntius conchonius* at various dosage levels is explained in detailed. It was found that fecundity differed depending on the dose of *Natrum muriaticum* used. The treated fishes produced the highest and lowest number of eggs, respectively, at 0.02 percent and 0.08 percent Sudha *et al.* (2015) [86]. An in-vivo experiment was conducted to determine the prophylactic effectiveness of *streptococcinum* 30c, *Aconitum napellus* 30c, and

Crotalus horridus 30c in tilapia fish infected with *Streptococcus agalactiae*. *Crotalus horridus* has shown effective antibacterial action against *Streptococcus agalactiae* John *et al.* (2019) [38]. In their research the effect of homeopathy complex on fatty acids in muscle and performance of the Nile tilapia (*Oreochromis niloticus*) was evaluated and these will be lower performance of homeopathy treated fish when compared with control group Andretto *et al.* (2014) [6]. In their study they have analysed the effects of *Streptococcinum*, *Hepar Sulfur*, *Rosmarinus officinalis* and erythromycin on cultured rainbow trout (*Oncorhynchus mykiss*) with experimental streptococcosis was performed and the survival rate will be better when compared with chemical drug and control group Salehi *et al.* (2015) [76]. The performance, occurrence of ectoparasites, and morpho-functional response of the liver and branchiae of Nile tilapia (*Oreochromis niloticus*) reared on fish meal with varying concentrations of the homeopathy compound Homeopatila 100 were investigated. The addition of Homeopatila 100 to the diet of Nile tilapia juveniles at a concentration of 40 mL per kg/meal could be a valuable measure in fish breeding Braccini *et al.* (2013) [15]. The *in vitro* treatment of RBCs with potentized medicines like *Merc cor* 30c and *Nux vom* 30c resulted in increased water penetration through the red cells. When the fish were pretreated *in vivo* with *Nux vom* 30c, the influx of water through the cells was increased even more Sukul *et al.* (2003) [87].

The effect of homeopathy treatments made from *Vibrio parahaemolyticus* and *Vibrio alginolyticus* (H1), as well as commercial homeopathy medications *Phosphoricum acidum* and *Silicea terra* (H2), on the immune and antioxidant responses in *Seriola rivoliana* juveniles challenged with *Vibrio parahaemolyticus* under standard culture conditions Mazón-Suástegui *et al.* (2019) [54]. The greatest survival recorded in animals that received treatments TH2, TH3 and TH4 after *V. parahaemolyticus* challenge indicated a protector effect, which shows that the homeopathy medications assessed could be an alternative tool for controlling bacterial diseases in shrimp farming. Homeopathy medicine increased survival and immunity of the shrimp *L. vannamei* juveniles when infected with *V. parahaemolyticus* Mazón-Suástegui *et al.* (2018) [52].

The researchers have found that the tested combination homeopathy medicines exhibited antiviral efficacy against the BHV-1 and BVDV-1 viruses *in vitro* and *in vivo* in animal virus Nefedchenko *et al.* (2015) [62]. *Influenzinum* RC, a biotherapy made from infectious influenza A virus in 30dH dilution, was developed in their research. In MDCK and J774G8 cell lines, the antiviral effects of this homeopathy medicine, as well as related molecular and biochemical features, were investigated. The impact of this biotherapy on J774's cytokine output G8 macrophages were studied, and J774 caused an increase in TNF α production. The presence of G8 macrophages suggests that the biotherapy has a stimulatory effect on these cells Siqueira *et al.* (2013) [83]. *Crotalus horridus* appears to be an effective and alternative in the event of ehrlichiosis, based on the findings of this study. On the seventh day after therapy, temperature, pulse, and respiration all decreased significantly. After treatment, the clinical symptoms also improved. All of the sick animals were more attentive and had a better appetite. After *Crotalus horridus* treatment, haemato-biochemical abnormalities caused by ehrlichiosis-

infected dogs restored to normal Tungnunga *et al.* (2016) [92]. The antipyretic effects of the homeopathy medications *Bell* and *Pyro* were explored in this research (200c and 1000c). When compared to paracetamol and *Bell*, *pyro* potencies (200c, 1000c) demonstrated a significant reduction in fever and more notable antipyretic effect against *S. cerevisiae*-induced fever. *Pyro* potencies have been shown to reduce fever by inhibiting cytokine induction and thereby preventing prostaglandin production Ahmad *et al.* (2019) [4]. When compared to the vehicle-treated group of animals, the rectal temperature of all the animals treated with *Aconite*, *Arnica*, *Belladonna*, and *Pyrogenium* did not lower with time Gosavi *et al.* (2010) [30]. The effects of *A. napellus* should be studied in a variety of fever models, particularly in the stress-induced hyperpyrexia model. The results of this study show that an ultrahigh dilution of the homeopathy drug *A. napellus* can produce antipyretic effect in rabbits with Baker's yeast-induced fever Ahmad *et al.* (2019) [4]. According to the findings, homeopathy medicines including *Arsenic album*, *Antimonium crudum*, *Hepar sulphur*, and *Silicea* have antibacterial activity as shown by Agar well diffusion assays, Minimum Inhibitory Concentration, and bactericidal studies and the result shows that the homeopathy medicines have anti-bacterial activity against *Staphylococcus epidermidis* Pasalkar *et al.* (2019) [66]. When the virus and drugs were given together or at different intervals post infection, the results of antiviral activity of homeopathy drugs against CEV of fowls clearly show that five drugs in their potencies, such as *Typhoidinum* 200, *Hydrophobinum* 1000, *Tuberculinum* 1000, *Nux vomica* 200, and *Malandrinum* 1000, completely inhibited viral lesions in the chorio-allantoic membranes of developing chick embryos. Homeopathy treatments have immediate antiviral effects, and they are effective whether taken before, subsequently, separately, or at intervals during a viral infection. These medications also developed an antiviral component, which may be interferon-like or something else new Singh *et al.* (1985) [82]. In their research they have concluded that *Bothrops Lanceolatus* is effective in treating coronary artery disease Panicker *et al.* (2021) [65].

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