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COMPARATIVE ANALYSIS OF HERBAL TREATMENTS OF *AEROMONAS HYDROPHILA*: A FISH PATHOGEN

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ABSTRACT

One of the main obstacles to sustainable animal production, particularly in aquaculture, is disease, which can result in severe financial damage. A common, free-living, gram-negative bacterium known as *Aeromonas hydrophila* causes a variety of illnesses, including epizootic ulcerative syndrome and aeromonad septicemia, in aquatic settings with a widespread occurrence. Several farmed fish have been killed as a result of this parasitic disease. Various chemotherapeutic treatments have historically been employed to cure it, but due to their lingering effects and other adverse impacts, such as antibiotic resistance and destruction of the environment, they cannot be advised. For millions of years, people have employed plants and other natural resources to treat a wide range of human issues. As a result, there has been a lot of interest in using these plants in aquaculture as an alternative to chemotherapies and antibiotics. Since they are inexpensive, easy to prepare, and don't have a lot of harmful effects on the environment or animals, these plants are gaining more and more attention around the world. Any part of the plant, including the roots, leaves, seeds, flowers, or extract chemicals, may be employed. Plants primarily function as immune system boosters and growth regulators, acting as an antimicrobial to the host immune response. This review is largely concerned with a variety of plants, such as herbs, seeds, and spices, in a variety of forms, such as crude, extracts, blended components, and bioactive elements. These plants are used as immunomodulators to prevent and control *Aeromonas hydrophila* in fish by significantly boosting their immune systems. The in vivo effects of several herbs and their constituents on the immune systems of various fish species as well as defense against a dangerous microbe *Aeromonas hydrophila are* addressed in this review.

Keywords: *Aeromonas hydrophila*; Antibacterial; Chemotherapy; Herbal; Immunity

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INTRODUCTION

Many developed and emerging nations are seeing substantial growth in the industrial aquaculture sector. It is a cost-effective protein source for feeding the world's population (Kaleo et al., 2019). During the last three decades, it has become the most emerging food-producing sector, contributing considerably to worldwide food supply, food security, and national economic growth (Sumon et al., 2018). It contributes significantly to development in terms of employment, employing over 41 million people worldwide (Gjedrem et al., 2012) Allison et al. (2012) reported the information about fisheries that provide job opportunities throughout the world estimated at 660–880 million people. Aquaculture is one of the fastest-growing businesses for the production of animal food due to the increase in global fish consumption in recent decades (Tadese et al., 2020). On a worldwide basis, aquaculture accounts for around 26% of all fish catches (Anderson, 2002). Fish is an essential element of the diet and is the source of nutrition. Aquaculture will be essential to address rising fish demand, which will be amplified by population expansion, increased wages, and urbanization (Gjedrem et al., 2012). Fish accounts for approximately 16% of all animal protein consumed by humans worldwide (Pradeepkiran, 2019). According to (FAO, 2012) fish is high in protein and provides vitamins and other elements that are important for human health. It accounts for around 16.6 percent of world's animal protein source. Fish provides 50% of the protein consumed in some countries like Bangladesh and Indonesia (Rehman et al., 2019).

Bacterial diseases have a negative impact on reproduction, growth, and productivity, are regrettably one of the significant issue in aquaculture (Elkamel and Mosaad, 2012). It induces a partial or complete reduction of fish output (Bondad-Reantaso et al., 2005). Many farmers across the world are still concerned about infectious diseases caused by bacteria in aquaculture systems because these can potentially result in significant financial losses (Sheikhlar et al., 2017). Aeromonas species are significant bacterial pathogens. The bacteria is currently viewed as one of the key issues in aquaculture, producing significant financial losses in fish farming globally (Lin et al., 2009).

For the treatment of these aquatic species, several medications and chemicals are recommended. Farmers utilize a variety of chemicals or antibiotics in fisheries (Tadese et al., 2020). However as a result of their continued use, pathogenic bacterial species become more resistant, their residues in fish tissues have increased, and the environment has become more contaminated (Abdel-Latif et al., 2020). The preferred way for controlling disease was the use of antibiotics and chemotherapy; unfortunately, the overemployment of chemotherapeutics, particularly antibiotics, has led to the emergence of numerous antibiotic-resistant bacteria (Elkamel and Mosaad, 2012). Alternatives to fisheries should be affordable, eco-friendly, and derived from natural sources. The use of phytogenics (plant derivatives), their extraction, and phytochemicals to boost fish immunity against bacterial illnesses has been one of these possibilities (Abdel-Latif et al., 2020). This study aims to review the role of plants in aquaculture in terms of protecting and controlling *A. hydrophila* infections in various fish species as well as stimulating the fish immune system.

Problems in aquaculture industry

The demand for fish and the strain on aquaculture to produce it has resulted in the widespread adoption of strict fish husbandry. The fact that all these production techniques produce substantially more per unit area due to higher stocking densities is one of the key components encouraging fish producers to use them. Contrarily, high growing conditions in intensive fish culture systems have resulted in several drawbacks, such as stress, which has been linked to poor fish performance, poor digestion and feed utilization, altered physiological functions, increased risk of disease, poor fish carcass quality, and in some extreme cases, death. The treatment of these stress-related disorders continues to be difficult, particularly in the top-producing nations for fish farming (Asians) (Gabriel et al., 2015). The aquaculture sector faces significant obstacles as a result of infectious illnesses (Adams et al., 2008). Some pathogenic microbes cause financial loss in the aquaculture business (Bilen et al., 2011). Numerous variables, such as overpopulation, mishandling, heat, poor water quality, and inadequate nutrition, cause fish to become stressed and immunosuppressed, which increases their vulnerability to infectious illnesses (Reverter et al., 2014).

The major problem is *A. hydrophila*. They are non-spore-forming, facultatively anaerobic, tiny, gram-negative, flagellated bacilli with a single flagellum that can ferment glucose either without or with the generation of gas. They have a length of 1.0–3.5 μm and a diameter of 0.3–1.0 μm. They are widespread and native to watery habitats. The genus Aeromonas, one of the 14 species that make up the family Aeromonadaceae, is known to infect fish, reptiles, amphibians, and people among its 14 members. *A. hydrophila* is the source of some diseases that can transfer from animals to people as well as from persons to animals (Pachanawan et al., 2008). This species is frequently found in freshwater, and it is a typical pathogen that threatens aquatic life (Khushiramani et al., 2008).

Since this gram-negative bacterium can survive in both oxygenated and anaerobic environments and has a diverse pathogenicity to numerous organs, it poses a specific threat to several freshwater fish (Devi et al., 2016). Sometimes illness seems to be caused by infectious agents in fish caused by environmental strains of *A. hydrophila*. However, in some instances, the major pathogens of *A. hydrophila* causing fish disease can be readily distinguished. A significant disease that affects practically all animal taxa, fish intestinal inflammatory response causes substantial deaths in a variety of fish species at various growth stages (Tadese et al., 2020). *A. hydrophila* destroys fish skin after entering the body through the gills and causes effects such as tissue enlargement, dropsy, red blisters, necrosis, ulcers, feather rot, and hemorrhagic septicemia, according to various investigations on fish (Awad and Austin, 2010).

Therefore, there are certain approaches, such as the use of immunomodulators and immunization to protect fish from pathogens to control *A. hydrophila* infection. Conversely, numerous drawbacks in the cultured fish sector prevent the use of vaccines and immunostimulants. Losses associated with vaccination administration, application challenges, and the extremely expensive prices of immunostimulants are all significant contributing causes (Bilen et al., 2011).

Antibiotics and chemotherapy

The best method for controlling fish infections is now known to be antibiotics. Antibiotics and other chemotherapeutic medications have been used by fish farmers for years to treat stress-related illnesses, particularly in commercial fish farming. The capacity of antibiotics to boost growth, improve feed conversion efficiency, and stop the propagation of infections makes them successful in aquaculture and other farming industries, including poultry and animals (Gabriel et al., 2015). However, a growing worry in aquaculture is the resistance to antibiotics for aquatic diseases (Basha et al., 2013). The occurrence of leftover antibiotics in processed fish and shellfish is yet another issue brought on by the overuse of antibiotics in industrial aquaculture (Oliveira et al., 2020). Because of this issue, users of fish may unknowingly consume antibiotics, which may change their normal flora and make them more susceptible to bacterial illnesses and the selection of antibiotic-resistant bacteria (McDermott et al., 2002).

Chemotherapy is frequently utilized to treat infectious bacterial, fungal, and parasite disorders. It has been applied to the treatment of bacterial and parasite illnesses in grouper. Experience has shown, however, that there are issues with chemical therapies for fish ailments; in certain instances, the treatments might be hazardous due to the stress they generate. There have been some instances of drug overdoses in Singapore that have killed fish and had other negative impacts. Significant gill damage results from formalin overdose. Repeated nitrofurazone treatments cause ulcerative dermatitis, which kills fish. The use of chemotherapy has led to issues with toxicity, resistance, residues, and perhaps some negative effects on the environment and public health. They can be expensive, and their effectiveness in open-water systems, where specific aquatic conditions exist, is still debatable. The use of chemicals in the treatment of health issues has become more challenging as a result of producers being misinformed by feed and chemical companies about the usage of antibiotics and other treatments (Punitha et al., 2008).

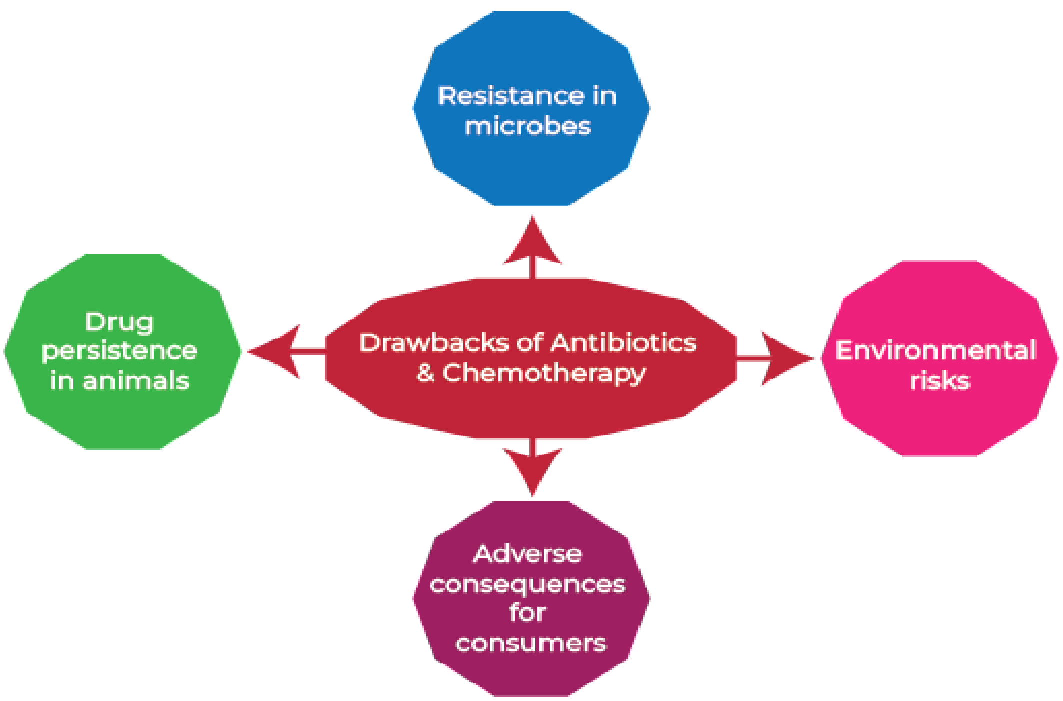

Figure 1. Graphical representation of drawbacks of antibiotics and chemotherapy.

Problem with antibiotics and chemotherapy

However, continuous and inappropriate use of chemotherapies causes a rise in resistance to dangerous bacteria, environmental risks, and adverse consequences for consumers (Magouz et al., 2021). The constant use of antibiotics as well as other chemicals has several drawbacks, including the risk of bacteria becoming resistant to treatment, the issue of drug persistence in animals treated with them, implications for human health, and environmental contamination (Gabriel et al., 2015). The usage of antibiotics has increased the resistance to antibiotics of environmental microorganisms and fish diseases. The growth of antibiotic-resistant fish illnesses endangers the effectiveness of antibiotics in aquaculture (Cabello, 2006). When antimicrobial medicines are released without restriction into the surrounding water to treat bacterial fish illnesses, the aquaculture ecosystem is negatively impacted. Because of its detrimental effects, using antibiotics and other chemotherapeutics to treat disease has been deemed unacceptable (Thiyagarajan et al., 2014). Vaccination is a further effective method of disease outbreak prevention (Austin et al., 2007). However, vaccines are typically too expensive and challenging to use widely in fish farms, besides the fact that a single vaccine only effectively combats a single type of microbe (Plant and LaPatra, 2011).

Due to the fast-rising emergence of resistance to currently available treatments, conventional antibacterial treatment is currently experiencing a crisis. Such resistance affects every aspect of chemotherapy (Punitha et al., 2008)**.** Along with the spectacular industrial development, numerous actions that can be detrimental to both human and animal health have been seen (Goldburg and Naylor, 2005) include releasing a lot of veterinary medications into the surroundings.

The solution to antibiotics

Several countries throughout the world have severe laws that restrict the use of antibiotics in animal agriculture. Therefore, it is vital to look for antibiotic alternatives that might be employed for improved growth efficiency, illness reduction, and ultimately better output in intensive fish production technologies (Gabriel et al., 2015)**.**

Drug-resistant bacteria may be treated with some extracted chemotherapeutic medicines from plants. Antibiotic replacements that have been successfully used include volatile oils, tannins, phenolic chemicals, saponins, alkaloids, polysaccharides, and polypeptides. By testing plant extracts and natural products for antimicrobial activity, researchers have shown that medicinal plants are a potential source of new anti-infective drugs, as well as through the use of natural ingredients in drug development for main lead substances (Punitha et al., 2008). Several number of plant compounds with antibacterial, antistress, immunomodulatory, and growth-promoting properties had a big impact on fish culture (Punitha et al., 2008)**.**

Using organic, inorganic, or synthesized substances as immunostimulants has been utilized as a superior alternative to antibiotics to treat fish infections since the fish's defense mechanism against microbes is very significant (Cuesta et al., 2004)**.** Research on modifying fish's non-specific immune system for both therapeutic and preventive objectives has increased during the past ten years (Elkamel and Mosaad, 2012)**.** Numerous other immunostimulants have so far been discovered to work in various species of fish. Over the past 10 years, organic fish culture has gained popularity, which has increased interest in natural immunostimulants. Several researchers discovered that fish fed with natural immunostimulants had an improved immune system (Bilen et al., 2011)**.**

Use of herbal products in aquaculture

Reducing the use of chemical treatments and their detrimental effects on fish and the environment requires the use of functional meals, as well as lowering production costs and obtaining more environmentally friendly aquaculture output (Can et al., 2012).

To maintain species health and promote optimal growth and productivity, aquaculture techniques need the use of high-quality feeds with a high crude protein content. These diets should also contain supplemental enhancers (Ayoola et al., 2013) The most effective feed additions to replace antibiotics are herbs (Meng et al., 2021). Aquaculture has actively researched appropriate nutritional immune enhancers, including as probiotics, functional carbohydrates, and other botanicals, to address different issues (Chen et al., 2016).

Plants and herbs are being used in aquaculture with greater success since they are affordable, environmentally beneficial, and have minimal unfavorable effects. It appears that conventional natural supplements can boost the immune system. They not only play a key part in fish culture but are also widely dispersed throughout Asia and safe for humans to ingest. Numerous studies have shown that using herbal supplements helped fish produce more and protected them from sickness. Additionally, plant medications are readily available locally, easily biodegradable, and affordable, and their extracts are simple to manufacture. Natural herbs continue to be widely utilized in their original form and have been crucial in the creation of modern medicine (Thiyagarajan et al., 2014). Immunostimulants improve innate immune systems and also the acquired immune system to promote resistance to contagious diseases. Numerous immunostimulants have been discovered to work in fish (Devi et al., 2016; Yin et al., 2008).

Herbal treatment in different fish species to treat *A. hydrophila*

Solid possible replacement materials are a crucial choice for steady and effective fish farming work. So natural products have received the most attention possible. The use of herbal items is the most significant natural product. They come from a variety of plant parts, including seeds, flowers, buds, fruits, and leaves (Baba et al., 2016). These plants can be utilized in their unprocessed, powdered state, or their various extracts can be employed to boost the resistance and survival of various fish species (Figure 2).

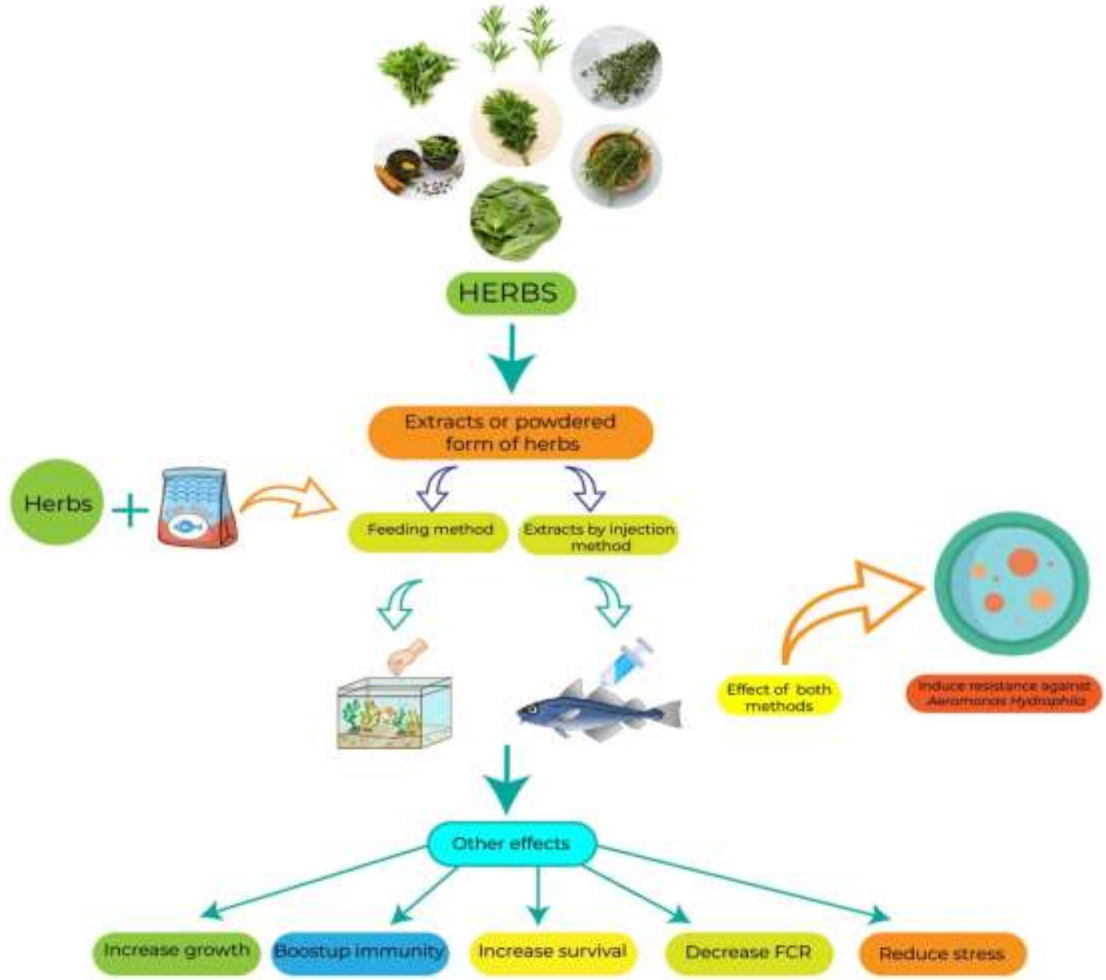

Figure 2. Graphical representation of application and effect of different herbs in fish to treat *A. hydrophila.*

Herbal treatment in Nile tilapia

Immunological effects

Ethanol extracts of *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber zerumbet* increased the nonspecific immunity of tilapia by increasing the total leukocyte and phagocytic index. Ethanol extracts of these herbs maintain the blood parameters (He, Hb, and TE) of Nile tilapia (Hardi et al., 2017). They act as an antibacterial and immunostimulant substance for fish. They increase the tilapia's leukocyte count (Hardi *et al*., 2017). It was also looked into if the addition of black cumin seeds, Nigella sativa, or Bacillus subtilis PB6 (CloSTAT) to feed would affect the Nile tilapia's immune system. The results showed that fish fed either a mixed diet or nigella considerably increased in white blood cells while fish fed either diet significantly increased in serum globulins. The combination ration-fed fish had significantly higher phagocytic activity and indices than the control groups (Elkamel and Mosaad, 2012).

Chinese herbs like *Lonicera japonica* and *Ganoderma lucidum* influence the fish's general immunological response. Increased phagocytosis by blood phagocytic cells after feeding tilapia Ganoderma and Lonicera separately or together (Yin et al., 2008). Two Chinese medicinal herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron are operative in the non-specific immune response of Nile tilapia. According to the study's findings, feeding tilapia with two herbs, either separately or together, greatly increased the blood phagocytic cells' phagocytic and respiratory burst activity. On the plasma lysozyme level, they had a mild impact (Ardó et al., 2008). Tilapia By 1 and 2 days after injection, having the hot-water extract of *Toona sinensis* at either dose markedly boosted respiratory burst, phagocytic activity, and lysozyme activity toward *A. hydrophila* (Wu et al., 2010). The effects of a Chinese herbal mixture composed of astragalus, angelica, hawthorn, Licorice root and honeysuckle (0, 0.5%, 1.0%, 1.5% or 2.0% w/w) for 1 month stimulated lysozyme, SOD and POD activity in serum, induced TNF-α and IL-1β mRNA expression in head kidney and spleen, but reduced serum MDA content (Tang et al., 2014). On Nile tilapia, the immune-stimulating effects of the leaves of Moringa (*Moringa oleifera*), Rosemary (*Rosmarinus officinalis*), and Turmeric (*Curcuma longa*) were evaluated. The *C. longa* containing group had higher serum lysozyme and respiratory burst activity than other groups. They also have biological effects as Nile tilapia treated with their leaves had higher blood albumin, globulin, and total protein levels than control fish (Ayoub et al., 2019).

Effect on *A. hydrophila*

The fish-fed isolates of Z. zerumbet at 200 and 2000 ppm exhibited 100 percent resistance to *A. hydrophila*. The percentages for fish fed with 600 and 900 ppm *B. pandurata* were 63% and 68%, respectively. In response to *Pseudomonas sp.* infection, the RPS of the fish given 900 ppm *S. ferox* was considerably higher than that of the fish given 400 ppm *S. ferox* (Hardi et al., 2017). The most potent antibacterial activity was seen in the alcoholic extract of *Psidium guajava* aerial parts. In vivo, tests revealed that fish diets containing either dried alcoholic extract of *P. guajava* leaf or its dry leaf powder reduced the mortality of tilapia infected with *A. hydrophila* with no discernible negative effects on the fish (Pachanawan et al., 2008). *B. pandurata* and *Z. zerumbet* extracts were utilized to effectively prevent *A. hydrophila* infection using the feed technique among the three delivery ways (injection method, feeding, and bath immersion), whereas *S. ferox* extract efficiently inhibited *Pseudomonas sp*. contamination by bath immersion (Hardi et al., 2017).

When taken separately or in combination, the Chinese herbs *Lonica japonica* and *Ganoderma lucidum* improved fish survival following *A. hydrophila* challenge. Fish fed Lonicera extract had a death rate of 43%, which was greater than that of control fish (58%), fish fed Ganoderma had a mortality rate of 30%, and fish fed a combination of two herbs had a mortality rate of just 21% (Yin et al., 2008). *Astragalus membranaceus* and *Lonicera japonica*, two Chinese medicinal herbs, along with boron, decreased the death rate after *A. hydrophila* inclusion. The group that consumed both herbs and boron together saw the lowest mortality. The mixture of boron and herbs could further boost the chances of diseased fish surviving (Ardó et al., 2008). Compared to the group given the control diet, the combination of different Chinese botaniclas including astragalus, hawthorn, licorice root, angelica, and honeysuckle with concentrations of 0 (control), 0.5%, 1.0%, 1.5%, or 2.0% demonstrated decreased mortalities after *A. hydrophila* infection (Tang et al., 2014).

Tilapia that had previously taken a hot-water extract of *T. sinensis* were then exposed to *A. hydrophila*. The survival of tilapia that received the hot-water extract of *T. sinensis* was considerably higher than that of fish that received phosphate-buffered saline and the control fish (Wu et al., 2010). The fish were given treatment pellets for 30 days that contained different concentrations including 0, 1.25, 2.5, 5, and 10 g of Zingiber officinale (ginger) powder per kg of feed. The fish received an intraperitoneal injection of 0.2 mL of an *A. hydrophila* suspension containing $5x10^7$ cfu/mL on day 31. The results demonstrated that supplementation of Z. officinale extract significantly influenced fish resistance to A. hydrophila infection when compared to control treatment. The maximum survival rate of fish was at 2.5 g of *Z. officinale* extract (Payung et al., 2017). Nile tilapia can be protected from *A. hydrophila* infection by including *M. oleifera*, *C. longa*, or *R. officinalis* in their diets; the RPS for the pretreated fish groups was 10, 75, 70, or 65%, respectively (Ayoub et al., 2019). The immune system of Nile tilapia can be modified using black cumin, CloSTAT, or maybe both to enhance disease resistance. Fish fed nigella or mixture rations and exposed to *A. hydrophila* had a much lower death rate compared to fish fed the standard diet (Elkamel and Mosaad, 2012) (Table 1).

Herbal treatment in *Labeo rohita*

Immunological effects

Fish were fed dietary quantities of powdered *Withania somnifera* (Ashwagandha) root for 42 days. The findings suggest that fish given W. somnifera root had higher levels of NBT, phagocytic activity, total immunoglobulin, and lysozyme activity than fish fed a normal diet (Sharma et al., 2010). The *Labeo rohita*'s immunity is stimulated by the inclusion of *Achyranthes aspera* seed in their diets. Comparing Achyranthes administered groups to the control group, it was shown that the synthesis of superoxide anion, serum bactericidal activity, lysozyme, ALP, serum protein, and albumin and globulin ratio were all increased. Although the SGOT and SGPT levels were higher in the control group, they were equivalent to those of the untreated control group in Achyranthes-treated groups (Rao et al., 2006).

Accelerated growth performance and non-specific immunological measures are both stimulated by the addition of andrographolide in the diet.

Table 1. Use of herbs in Nile Tilapia to treat *A. hydrophila*.

Pre infection									
Fish	Herb/plant	Part used	Conc. of plant	Route	Duration (Days)	Results			
						Growth parameters	Immunological parameters	Biochemical parameters	Hematological parameters
Oreochromis niloticus (Nile tilapia)	*Boesenbergia pandurate* (finger root), *Solanum ferox* (night shade), and *Zingiber zerumbet* (wild ginger)	Root (Extract)	(0.1 mL fish^{-1}) 600, 900, 200 ppm In feed and for 30 min in the bath.	IP injection, feeding, and bath immersion	14 D	-	↑Leukocytes.	-	-
Oreochromis niloticus (Nile tilapia)	*Boesenbergia pandurate* (finger root), *Solanum ferox* (night shade), and *Zingiber zerumbet* (wild ginger)	Root (extract)	600,900 ppm for B. pandurata, 400,900 ppm for S. ferox, 200,2000 ppm for Z. zerumbet	IP	21 D	-	↑leukocyte, ↑phagocytic index	-	↑HCT, ↑ Hb, ↑TE
Oreochromis niloticus (tilapia)	*Psidium guajava* (Guava)	Leaf	Diet 1 leaf powder 1:4 w/w. Diet 2 Ethanol extract1:24 w/w. Diet 3 oxy tetra- cycline 1:199 w/w	Diet	10 D	-	-	-	-
Oreochromis niloticus (Tilapia)	*Lonicera japonica* (Japanese honeysuckle) and *Ganoderma lucidum* (lingzhi mushroom)	-	1.0% of *L.japonica* and *G.luciduma* and 0.5% of the mixture of both.	Diet	21 D	-	↑phagocytosis, ↑lyz	-	-
Oreochromis niloticus (Nile tilapia)	*Astragalus membranaceus* and *Lonicera japonica* and boron	-	0.1% *Astragalus* and *Lonicera* and combination with 0.05 % of boron.	Diet	28 D	-	↑phagocytosis, ↑ respiratory burst activity ↑Lyz,	↑TP	-
Oreochromis mossanbicus (Tilapia)	*Toona sinensis* (red toon)	Leaf	(10µ L)4 or 8 µg g^{-1}	Injection in caudal vein	7 D	-	↑respiratory burst. ↑phagocytosis ↑lyz	-	-
Oreochromis	Chinese herbal	-	0.5, 1.0, 1.5 and	Diet	30 D	-	↑LYZ	-	-

niloticus (GIFT)	mixture		2.0%				↑SOD ↑POD ↓MDA		
O. niloticus (Nile tilapia)	*Zingiber officinale* (ginger)	Root powder	1.25, 2.5, 5, 10g powder/kg	diet	30 D	-	-	-	-
O. niloticus (Nile tilapia)	*Moringa oleifera* (Moringa), *Rosmarinus officinalis*(Rosemar) *and Curcuma longa* (Turmeric)	Leaf	1 %	diet	60 D	↑SGR	↑Lyz, ↑respiratory burst	↑TP, ↑albumin ↑globulin	-
Oreochromis Niloticus (Nile tilapia)	*Nigella sativa* (black cumin), *Bacillus subtilis*	seed	3%	diet	30 D	-	Phagocytic	TP, globulin	↑WBC

Post-infection

Pathogen	Conc. of pathogen	Route	Duration (Days)	Results	References
A. hydrophila and *Pseudomonas* sp	(0.1 ml)10^{10} CFU mL^{-1}	IM	14D	The extracts of B. pandurata and Z. zerumbet have an effective influence on preventing A. hydrophila infection by the feeding method, while S. ferox extract has a significant effect on preventing Pseudomonas sp. infection through bath immersion.	(Hardi *et al*., 2017)
A. hydrophila and *Pseudomonas* sp	0.1m L (10^{10} CFU mL^{-1})	IM	14D	200 and 2000 ppm *Z. zerumbet* extracts were 100% effective against *A. hydrophila*. 900 ppm S. ferox was effective against *Pseudomonas* sp.	(Hardi *et al*., 2017)
A. hydrophila	50µL (3.44×10^{6} CFU/ml)	IP	14 D	↑survival ↓ mortality	(Pachanawan *et al*., 2008)
A. hydrophila	0.1 ml (3 x 10^{6} cells/ml)	IP	10 D	↑survival	(Yin *et al*., 2008)
A. hydrophila	0.1 mL(5×10^{7} CFU/ml)	IP	10 D	↑ survival	(Ardó *et al*., 2008)
A. hydrophila	(10 µ L)5×10^{7} cfu mL-^{1}	2nd day IP	7 D	↑ survival	(Wu *et al*., 2010)
	0.2 ml (1.0×10^{8} CFU/ ml)	IP	10 D	↑survival	(Tang *et al*., 2014)
A. hydrophila	0.2 mL (5x10^{7} cfu/mL)	IP	8 D	↑survival	(Payung *et al*., 2017)
A. hydrophila	0.2mL (1.5 × 108 CFU/ml)	IP	10 D	↑survival	(Ayoub et al., 2019)
A. hydrophila	1.0×10^{7} cfu/ml	immersion	30 min	↑survival	(Elkamel and Mosaad, 2012)

When compared to the control group, fish-fed andrographolide displayed a significantly higher level of NBT, and the activity of myeloperoxidase, phagocytic, serum lysozyme, and serum antiprotease activity were all increased. Fish treated with andrographolide showed significantly different weight gains, specific growth rates, FCR, and protein efficiency ratios from controls (Basha et al., 2013). The immunological characteristics of *Labeo rohita* against the *A. hydrophila* infestation are greatly influenced by *Ocimum sanctum Linn* leaf. It increased the generation of superoxide anion, total immunoglobulin in plasma, lysozyme activity, total protein in serum, total RBC, WBC numbers, and hemoglobin concentration as compared to control (Das et al., 2015).

Effect on *A. hydrophila*

W. somnifera consumed by diet provided noticeably greater defense against *A. hydrophila* infection than the control. The experimental diet showed a higher rate of survival as compared to the control (Sharma et al., 2010). *L. rohita*'s resistance to infection is increased by *Achyranthes Aspera*. Up to day 9 after infection, the control group (77%) had higher overall mortalities. With increasing doses of Achyranthes, this gradually declined, with 66% morbidity in the 0.01% group, 57% death in the 0.1% group, and 28% deaths in the 0.5% group (Rao *et al*., 2006). In *L. rohita* finerlings, the inclusion of andrographolide with 0.10% concentration demonstrated significantly higher RPS (74.06%) against *A. hydrophila* disease than control. and improved disease resistance in *L. rohita* fingerlings infected with *A. hydrophila* (Basha et al., 2013).

L. rohita becomes more resistant to *A. Hydrophila* when *O. sanctum* leaf extract is used. Compared to the control, 0.2% dietary *O. sanctum* extracts significantly increased protective relative percentage survival against the discussed infection (Das et al., 2015) (Table 2).

Herbal treatment in *Cyprinus carpio* (common carp)

Immunological effects

All of the *Aeromonas* sp. isolates were reported to be inhibited in growth by aerial extracts of *Spondias pinnata*, *Eugenia caryophyllus*, and *Terminalia chebula*. This investigation also discovered that numerous antibiotic-resistant cultures were responsive to various herb extracts (Rahman and Hossain, 2010). The dietary supplementation of origanum essential oil substantially enhanced the activities of hepatic superoxide dismutase and catalase. The OEO extracted from *Origanum vulgare* has great effects on antioxidants, immunity conditions, and expression of immune-related genes in common carp. The optimal level of this oil in the diet is 15 g/kg. Hepatic malonaldehyde concentrations, though, sharply dropped. Dietary OEO raised serum lysozyme activity, phagocytic activity, and phagocytic index amounts in a dose-dependent manner, with the maximum values occurring at 15-20 g OEO/kg diet. Additionally, dietary OEO markedly increased the relative expression of hepatic IL-1β and IL-10 genes (Abdel-Latif et al., 2020).

Henna, or *Lawsonia inermis*, has been studied in common carp for its immunomodulatory characteristics. Different concentrations of methanolic soluble fractions of *L. inermis* were intraperitoneally administered to the fish. The extract increased various non-specific immunological parameters, including serum lysozyme and bactericidal interaction, phagocytic activity and respiratory burst action, leucocyte number, lymphocyte, monocyte, and neutrophil count, considerably at doses of 60 and 600 mg kg-1 body weight (Soltanian and Fereidouni, 2016). *Rehmannia glutinosa* appears to be a good feed addition for *C. carpio* in aquaculture, as it may speed up growth and improve immunological function in common carp. *Rehmannia glutinosa* root powder, prepared root powder, and dried root extract are all examples of this. The findings showed that the treated groups' growth performance significantly improved as compared to the control. In all trials supplemented RG, the non-specific immunological parameters like the lysozyme and leukocyte phagocytic activity were also enhanced (Wang et al., 2015).

Fish-fed diets containing a multivitamin diet and *Epilobium hirsutum* harvest did not differ significantly (P>0.05) from control in terms of specific growth rates, feed conversion ratios, or survivability. Common carp-fed meals with various levels of plant extract did not significantly differ in terms of moisture, protein, crude fat, or ash content (P>0.05). White blood cells were significantly enhanced in both the infected and uninfected groups when compared to the control group, according to hematological parameters (Pakravan et al., 2012). The majority of bacteria are susceptible to the bactericidal effects of *Litsea cubeba* leaf oil (*A. hydrophila, Vibrio furnissii, Edwarsiella tarda, Vibrio parahaemolyticus, Escherichia coli, Streptococcus garvieae, Salmonella Typhimurium*). The 21-day leaf powder supplemented diets showed common carp nonspecific immunity. *L. cubeba* supplementation enhanced weight gain, SGR, and FCR in fish. A significant difference from the control occurred at the highest dose (8%) used. All of the groups treated experienced a considerable rise in plasma lysozyme. For the supplemented groups with 4 and 8% plant powder, hemolysis activity was increased. Only 8% concentration of the dose did antibacterial activity significantly increase (Nguyen et al., 2016).

Table 2. Use of herbs in *Labeo rohita* to treat *A.hydrophila*.

Pre infection									
Fish	Herb/plant	Part used	Conc. of plant	Route	Duration (Days)	Results			
						Growth parameters	Immunological parameters	biochemical parameters	Hematological parameters
Labeo rohita (Indian major carp)	*Withania somnifera* (Ashw agandha)	Root powder	1,2,3 g kg^{-1}	Feed	42 D	-	↑ NBT, ↑Phagocyte , ↑Ig, ↑lyz	-	-
Labeo rohita	Andrographolide substance	-	0.00, 0.05, 0.10, 0.20, 0.40 and 0.80%	Diet	42 D	↑WG, ↑FCR, ↑SGR	↑NBT, ↑myeloperoxidase, ↑phagocytosis, ↑lyz, ↑antiprotease	-	-
Labeo rohita	*Ocimum sanctum* Linn (Tulsi)	Leaf	0.0, 0.05, 0.1, 0.2, 0.5and 1%	Diet	42 D	-	↑NBT, ↑lyz, ↑Ig ↑superoxide anion,	↓Glu, ↑TP, ↑albumin, ↑Globulin, ↑A\G	↑RBC, ↑WBC, ↑Hb
Labeo rohita, rohu fingerlings	*Achyranths aspera* (Chaff-flower)	Seed	0.01, 0.1 and 0.5%	Diet	14 D	↑GR, ↑FCR	↑Superoxide anion, ↑bactericidal activity, ↑lyz, ↑ALP, ↑protein, ↑ (A/G), ↓SGOT, ↓SGPT	-	-

Post-infection					
Pathogen	Conc. of pathogen	Route	Duration (Days)	Results	References
A. hydrophila	(100 µl)1× 10^{-7} cells ml^{-1}	IP	14D	↑survival	(Sharma *et al*., 2010)
A. hydrophila	0.2 ml (1.8× 10^{6} cfu m^{-1})	IP	14 D	↑survival	(Basha *et al*., 2013)
A. hydrophila	0.10mL (1×10^{7}cells mL^{-1})	IP	18 D	↑survival	(Das *et al*., 2013)
A. hydrophila	100 µL(3×10^{6} CFU)	IP	9 D	↑survival	(Rao *et al*., 2006)

Effect on *A. hydrophila*

Common carp's immune system and antioxidant status are both strengthened by dietary origanum essential oil. The 10-day overall mortality dramatically decreased after the *A. hydrophila* challenge; however, origanum essential oil delivery greatly raised the relative percent of common carp survivability in a dose-dependent manner (Abdel-Latif et al., 2020). Infectious illnesses in aquaculture can be effectively controlled by *L. inermis*. After treating common carp with *L. inermis*, disease resistance against *A. hydrophila* can be increased (Soltanian and Fereidouni, 2016).

Common carp had a much greater survival rate in the groups. When challenged with *A. hydrophila*, dried root powder of *Rehmannia glutinosa*, prepared root powder of Rehmannia, dried root extract of Rehmannia, and prepared Rehmannia root extract all performed better than the control (Wang et al., 2015). Fish fed a meal containing Epilobium hirsutum extract experienced considerably lower *A. hydrophila*-induced fish mortality than fish fed a control diet. The group fed a diet containing 3% plant extract experienced the lowest mortality rate (Pakravan et al., 2012). An assortment of 4-8% *L. cubeba* leaf powder supplementation diets can be beneficial for aquaculture to lessen the load of antibiotics and the effects of diseases brought on *A. hydrophila*. Fish-administered doses of 4 and 8% *L. cubeba* performed significantly better after infection with *A. hydrophila* than the other groups (Nguyen et al., 2016) (Table 3).

Use of herbs to treat Aeromonas specie in rainbow trout

Safflower extract dosages of 50 to 200 mg/kg did not significantly change the serum antibacterial activity toward *A. hydrophila* and *Y. ruckeri*, whereas injections of 100 mg/kg boosted the serum antibacterial resistance against *S. iniae* in rainbow trout (Zargari et al., 2018).

Rainbow trout mortality was reduced after an *A. hydrophila* challenge by feeding them 1% *Lupinus perennis*, *Mangifera indica*, or *Urtica dioica*, for 14 days. The treated group significantly outperformed the controls in terms of serum bactericidal activity, respiratory burst, and lysozyme activity. Stinging nettle use resulted in the greatest hematocrit and haemoglobin values, while the use of lupin and mango increased the number of RBC and WBC in recipient fish. The control groups and those who consumed stinging nettle had the highest mean corpuscular volume and hemoglobin levels (Awad and Austin, 2010) (Table 4).

Herbal treatment in *Carassius auratus* (goldfish)

Azadirachtin exhibits higher NBT activity, protein profiles, serum lysozyme, leukocyte amounts, and resistance against *A. hydrophila* infection in goldfish. A unique promise for aquaculture is the modulation of immune responses utilizing active bio-ingredients as a potential prophylactic tool. The total erythrocyte number, total leukocyte quantity, Ig, total protein, activity, serum lysozyme function, and myeloperoxidase expression were all increased in fish-fed azadirachtin. Serum glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, and glucose levels of the blood were also discovered to be substantially higher in the treated group compared to the control groups. while packed cell volume and hemoglobin did not differ significantly (Kumar et al., 2013).

Herbal treatment in *Labeo victorianus* (Victoria Labeo)

Dietary therapy of stinging nettle enhanced biochemical, hematological, and immunological markers in juvenile and adult Labeo victorianus against *A. hydrophila*. Among some of the biochemical measures; plasma cortisol, glucose, triglyceride, and cholesterol were reduced while total protein and albumin in fish rose with the inclusion of a high dose of *U. dioica*. RBC, WBC counts, hematocrit, mean cell hemoglobin, mean cell hemoglobin concentration, and neutrophils were increased by using *U. dioica*. The main immunological measurements in adult and juvenile L. victorianus were serum immunoglobulins, lysozyme activity, and respiratory burst, and they all increased with increasing herbal incorporation of *U. dioica* in the diet. In comparison to A. hydrophila, dietary inclusion of *U. dioica* at 5% resulted in significantly greater relative percentage survival which was up to 95% (Ngugi et al., 2015) (Table 5).

Herbal treatment in African catfish (*Clarias gariepinus)*

Piper betle, *Tithonia diversifolia*, and *Psidium guajav* are three plants that can be utilized to combat *A. hydrophila* infection in fish. All three plant powders implanted in meal significantly reduced fish mortalities during experimental infection with *A. hydrophila*, even though the lowest inhibitory and bactericidal concentrations of acetone extracts were quite high., as evidenced by RPS ranging from 77 to 79%. In vitro, the three plant extracts had no effect when applied together. Although they increased RPS compared to controls, they were less effective at lowering mortalities than single plants. None of the plant-enriched diets had any noticeable effects on the quantity of blood cells or the formation of reactive oxygen species, except *P. betle*, which considerably increased ROS production following infection. However, the decrease in hematocrit following infection was greatly mitigated by both individual plants and their mixes. Overall, these results demonstrate that selecting plants for herbal therapy is challenging and may be influenced by a variety of in vivo conditions; as a result, one shouldn't rely exclusively on testing plants' antimicrobial activity (Nafiqoh et al., 2020) (Table 5).

Table 3. Use of herbs in Common Carp to treat *A. hydrophila*.

Pre infection									
Fish	Herb/plant	Part used	Conc. of plant	Route	Duration (Days)	Results			
						Growth parameters	Immunological parameters	biochemical parameters	Hematological parameters
Cyprinus carpio (common carp)	*Origanum vulgare*	Oil	5, 10, 15, and 20 g per kg	Diet	60 D	-	↑LYZ, ↑phagocytosis, ↑phagocytic index	-	-
(*Cyprinus carpio*) common carp	*Lawsonia inermis* (henna)	Leaf	6, 60 or 600 mg kg-1	IP Injection (200 µL)	The fish were bled 2 days before and 2, 4, 6, 8, and 10 days after treatment	-	↑NBT, ↑lyz, ↑phagocytosis, ↓Monocyte, ↓Granulocyte, ↑WBC's	↑TP, ↑globulin	Hb, Hct, and RBC values were not affected
(*Cyprinus carpio*) common carp	*Rehmannia glutinosa*	Root	2 DP, 4 DP, 2 PP, 4 PP, 0.5 DE, 1 DE, 0.5 PE, and 1% PE	Diet	80 D	↑SGR	↑LYZ, ↑phagocytosis	-	-
Cyprinusc arpio Somayeh (common carp)	*Epilobium hirsutum* (willow herb*)*	Aerial parts	0,0.5, 1.3,3% and 12% multivita min	Diet	60 D	SGR, and CF FCR showed no sig. diff.	↑ WBC's. RBCs,s, Hb, and HCT had no difference	-	↓RBC ↑WBC ↑HCT No diff. in Hb lymphocyte, monocyte, and neutrophil
Cyprinus carpio (common carp)	*Litsea cubeba* (aromatic litsea)	Leaf powder	2,4,8%	Feed	21 D	↑WG, ↑FCR ↑SGR,	↑LYZ, ↑Hemolytic activity, ↑Antibacterial activity	-	-

Post-infection					
Pathogen	Conc. of pathogen	Route	Duration (Days)	Results	References
A. hydrophila	(200 µL)1 × 10^6 CFU/mL	IP	10 D	↑survival	(Abdel-Latif *et al*., 2020)
A. hydrophila	0.1mL (1 × 10^8 cells/fis)	IP	15 D	↑survival	(Soltanian and Fereidouni, 2016)
A. hydrophila	4 × 107 CFU	IP	48 h	↑survival	(Wang *et al*., 2015)
A. hydrophila	3 ×10^8 CFU mL-^{1}	IP	30 D	↓ mortality in group fed diet 3% extract	(Pakravan *et al*., 2012)
A. hydrophila	(0.1 ml) 108 CFU ml^{-1}	IP	*21D*	↑survival	(Nguyen *et al*., 2016)

Table 4. Use of herbs in rainbow trout to treat *A. hydrophila.*

Pre infection									
Fish	Herb/plant	Part used	Conc. of plant	Route	Duration (Days)	Results			
						Growth parameters	Immunological parameters	biochemical parameters	Hematological parameters
*Oncorhynchus mykiss (*rainbow trout*)*	*Carthamus tinctorius* (safflower)	Flower	50, 100, 200 mg/kg of fish weight	Injection	7 D	-	-	-	-
Oncorhynchus Mykiss (rainbow trout)	*Urtica dioica* (nettle), *Mangifera Indica* (mango), *Lupinus perennis* (lupin)	Leaf, fruit, seed	1, 2%	Diet	14 D	-	↑Phagocytosis, ↑respiratory burst, ↑lyz, bactericidal, ↑myeloperoxidase, ↑antiprotease, ↑ a2-macroglobulin, ↑cytokines (IL-1b, IL-8, TGF-b)	-	↑RBC, ↑WBC, ↑Hct, ↑MCH, ↑MCV, ↑HB

Post-infection					
Pathogen	Conc. of pathogen	Route	Duration (Days)	Results	References
A. hydrophila, Streptococcus iniae, and Yersinia ruckeri		In vitro		Dose of 50-200 mg/kg didn't effective against A. hydrophila and Y. ruckeri, but 100 mg/kg against S. iniae,	(Zargari *et al*., 2018)
A. hydrophila	(0.1mL)10^7 cells mL^{-1}	IP	10 D	↑survival	(Awad & Austin, 2010)

Table 5. Use of herbs in different other species of fish to treat *A. hydrophila*.

Pre infection									
Fish	Herb/plant	Part used	Conc. of plant	Route	Duration (Days)	Results			
						Growth parameters	Immunological parameters	biochemical parameters	Hematological parameters
Carassius auratus (goldfish)	azadirachtin	-	0.1, 0.2, 0.4, 0.8 and 1.6%	Diet	28 D	-	↑NBT, ↑Myeloperoxidase, ↑LYZ, ↑phagocytosis, ↑Ig, ↑SGOT, ↑SGPT,	↑Glu, ↑TP, ↑Albumin, ↑globulin, ↓A\G	↑Erythrocyte, ↑Leucocyte,↑Hb, ↑PCV, ↑MCV,↑MCH,↑MCHC
Labeo victorianus (Victoria Labeo)	*U. dioica* (stinging nettle)	Leaf	0,1,2,5%	diet	30 D and 120 D on both juvenile and adults	↑SGR	↑Ig, ↑Lyz, ↑respiratory burst	↓cortisol, ↓Glu, ↓triglyceride, ↓cholesterol, ↑TP, ↑albumin	↑RBC, ↑WBC, ↑Htc, ↑MCH, ↑MCHC.
Clarias gariepinus	*Piper betle*, *Psidium guajava*,and *Tithonia diversifolia*	Leaf powder	80g /kg	diet	30 D	-	↑monocytes in fish fed with *T. divesifolia*	-	↓HCT fish fed with *P. betle*

Post-infection					
Pathogen	Conc. of pathogen	Route	Duration (Days)	Results	References
A. hydrophila	0.2 mL	IP	14 D	↑survival	(Kumar *et al*., 2013)
A. hydrophila	(0.10ml) 1×10^{7} CFU	IP	21 D	↑survival	(Ngugi *et al*., 2015)
A. hydrophila	107 CFU mL^{-1}	IM	10 D	↑survival	(Nafiqoh *et al*., 2020)

CONCLUSION AND RECOMMENDATIONS

In conclusion, supplementation of herbs in different fish species significantly improves the ability to resist against a fatal pathogen *A.hydrophila* by enhancing fish growth performance, and immunological, biochemical, and hematological parameters. The use of herbs in aquaculture may also reduce the cost of disease management by precluding the expenses experienced by the use of antibiotics, chemicals, and vaccinations. Our study provides a valuable basis for further treatment of *A.hydrophila* and facilitates husbandry for the economically valuable growth of different fish species. Further studies must be carried out to identify the exact mechanism of action of herbs with this pathogen, helping to answer how these herbs exactly work in aquaculture. Further studies are also needed to investigate the effect of all these herbs on different water parameters. It is important for farmers to use herbs for the economic benefits and for the long-term sustainability of aquaculture. Dealing with the *A. hydrophila* by using herbs rather than antibiotics or other chemotherapeutic treatments can have a significant impact on aquaculture as well as on the environment.

ABBREVIATIONS USED

WG =Weight gain, FCR= Feed conversion ratio ,SGR=Specific growth ratio ,HCT=hematocrit , TE=total erythrocyte , (A/G)=albumin globulin ratio ,ALP=alpha lipoic acid , TP=total protien, SGOT=Serum glutamate oxaloacetate transferase, SGPT=serum glutamate pyruvate transferase , SOD =Superoxide dismutase , POD=peroxidase , MDA=Malondialdehyde , DP= Dried rehmannia root powder, PP=prepared rehmannia root powder, DE =dried rehmannia root extract, PE =prepared rehmannia root extract, lyz=Lysozyme.

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