

Review Article

Overview of *Lemna* sp. in Aquaculture: Flavonoids for Boosting the Immune System in Fish

ABSTRACT

This study aims to provide information that the content of flavonoids in *Lemna* sp. can be an effort to control disease in fish. The research method used is a quantitative descriptive method. The conclusion of the research is to show the ability of *Lemna* sp. can be used in the prevention of disease in fish. *Lemna* sp. has immunomodulatory properties, namely the ability to increase phagocytosis so that it can increase the body's immunity in fish. Flavonoid content in *Lemna* sp. has antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties coupled with their capacity to modulate cellular enzyme function. Flavonoids also have the potential to stimulate the immune system

Keywords: Flavonoid, Immune System, Lemna sp., Fish

1. INTRODUCTION

In recent years, the importance of maintaining a strong immune system in fish has gained significant attention. With the rise of infectious diseases and environmental stressors, researchers have been exploring natural compounds that can enhance the immune response in fish. The disease is one of the factors that can inhibit fish farming, especially bacterial diseases. Apart from being a nuisance, this disease is generally caused by other organisms, feed, and environmental conditions that do not support fish survival. Fish diseases are divided into two, namely infectious diseases (bacteria, viruses, parasites, and fungi) and non-communicable diseases (stress, tumors, nutritional disorders, and feed trauma) [1].

Fish that are attacked by disease can be characterized by a decrease in the ability of fish to maintain normal physiological functions [2]. Therefore, an effort is needed to overcome the spread of disease in fish. Prevention is a preventive measure that can be carried out by providing adequate feed and providing immunostimulants [3]. Management of fish health by utilizing natural ingredients is an effective solution to dealing with sick fish. The use of natural ingredients is an alternative treatment that is cheap, effective, and natural [4].

Flavonoids are a group of plant compounds that have gained increasing attention in recent years due to their potential health benefits. Found in a variety of fruits, vegetables, and other plant-based foods, these natural compounds have been shown to possess powerful antioxidant and anti-inflammatory properties, making them a crucial component of a healthy diet. *Lemna* sp. is known to have several bioactive compounds and polysaccharide macromolecules that can function as immunomodulators to influence non-specific immune responses to increase the body's resistance to pathogens [5]. *Lemna* sp. contains vitamin E, carotenoids, and flavonoids which can be used as a source of antioxidants. has the potential to increase the body's resistance to fish because it contains carotenoids and antioxidant activity which is quite high [6]. In addition, *Lemna* sp. has immunomodulatory properties, namely the ability to increase phagocytosis.

Therefore, with the existence of disease problems in fish, it is necessary to use safe alternative materials to control fish diseases using natural ingredients. Availability of Lemna sp. The abundance of fish can be used as an effort to utilize natural ingredients and increase growth and the immune system in fish.

2. METHODS

The data obtained from the research results were analyzed using a quantitative descriptive method. The descriptive method aims to tell and interpret data relating to situations that occur in a systematic, factual and accurate manner regarding facts and relationships between variables to get the truth, while the quantitative method aims to raise facts, state variables and phenomena that are happening now and present them as they are[7].

3. FLAVONOIDS COMPOUNDS

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. Flavonoids are antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic coupled with their capacity to modulate cellular enzyme function [8]. Flavonoids are closely related to antioxidants because they have the ability to break down free radicals [9]. Further studies have shown that flavonoids also stimulate internal antioxidant enzymes, suppress enzymes related to the formation of free radicals, and bind metals [10].

4. FLAVONOIDS IN AQUACULTURE

Flavonoids are very effective antioxidants. Flavonoids also have the potential to stimulate the immune system, induce protective enzymes in the liver, or block damage to genetic material [11]. There is ample evidence showing that free radicals cause oxidative damage to lipids. Plant flavonoids may also reduce the risk of thrombosis by inhibiting platelet aggregation and adhesion. Flavonoids can inhibit enzymes involved in the oxidation of polyunsaturated fatty acids. Flavonoids inhibit platelet aggregation by mediating other enzyme systems. Its antioxidant properties directly participate in its antithrombotic action [12]. Flavonoids have the potential to play an important role in aquaculture. These compounds can be used to enhance the immune system of fish, which can help reduce the occurrence of diseases. Furthermore, flavonoids can be used to improve water quality, enhance growth and survival rates, and reduce stress. This highlights the potential of Lemna sp. as an aquaculture feed additive, as it is a rich source of flavonoids. With further research and development, Lemna sp. could become an important feed ingredient for fish farming.

5. FISH IMMUNITY SYSTEM

The immune system of fish is physiologically similar to that of higher vertebrates, although there are certain differences. Unlike higher vertebrates, fish are free-living organisms from the early embryonic life stages, relying on an innate immune system for survival [13]. These receptor proteins recognize molecular patterns typical of pathogenic microorganisms, including polysaccharides, lipopolysaccharide (LPS), bacterial peptidoglycan DNA, viral RNA, and other molecules not normally found on the surface of multicellular organisms. This response is divided into physical barrier, cellular immune response and humoral immune response. These immunological parameters include growth inhibitors, lytic enzymes, classical complement pathway, alternative and lectin pathways, lectins and precipitins (opsonins and primary lectins), antibodies, cytokines, chemokines, and antimicrobial peptides. Various internal and external factors affect the

parameters of the innate immune response. Temperature changes, stress management and density can have an inhibitory effect on this type of response, while various food additives and immunostimulants can enhance its effectiveness [14].

The immune system in fish is an immune system that involves self-defense mechanisms against foreign particles, especially disease-causing pathogens. If there is an infection of microorganisms in the fish's body by pathogens from types of bacteria, viruses, and parasites/fungi that enter the body, the fish or shrimp will respond with the body's defense system [15]. The immune system in fish is known to consist of an innate defense system or an innate/natural defense system that is non-specific and a specific immune system or an adaptive response [14]. It is said to be non-specific because it does not only respond to certain (non-specific) types of pathogens. This immune system has existed since birth/hatch, so it is said to be natural. The non-specific immune system in fish, for example, is physical (skin, mucus, scales), humoral (lysozyme, stomach acid, lactoferrin, complement, interferon), and cellular (phagocytes, NK cells) [16].

The non-specific defense systems in fish generally sweep adaptive responses, activate and determine the nature of adaptive responses and cooperate in maintaining homeostasis. The specific immune system is a mechanism of cooperation and interaction between phagocytes and lymphocytes. The emergence of a specific response starts from the work of phagocytic cells or macrophages or also called Antigen Presenting Cells (APC). This APC will then introduce it to specific immune cells, namely T cells and B cells. The adaptive or specific immune defense system will function properly when induced by exposure to pathogens or products derived from pathogens (for example LPS and vaccines). This specific defense system only reacts to certain antigens [17].

Fish have been known to rely more on the mechanisms of their non-specific/natural/innate immune system than on their specific immune system [18]. Non-specific defense is a very important body defense system in the fish immune system. In fish, the new immune response is fully formed when the fish are adults. Juvenile fish do not have a perfect specific immune response and rely on non-specific cell responses to defend against microbial infection. Non-specific defense is the main defense in fry and juvenile fish [19]. According to Buchmann (2014), immunostimulants are substances or drugs that can restore the disturbed immune system by stimulating and improving immune system function. Administration of immunostimulants can be administered through injection, feed (oral), and immersion [20].

5.1 Nonspecific Immunity

In fish, the innate response has been considered an essential component in combating pathogens due to limitations of the adaptive immune system, their poikilothermic nature, their limited repertoire of antibodies and the slow proliferation, maturation and memory of their lymphocytes [21]. It is commonly divided into three compartments: the epithelial/mucosal barrier, the humoral parameters and the cellular components. The epithelial and mucosal barrier of the skin, gills and alimentary tract is an extremely important disease barrier in fish, being constantly immersed in media containing potentially harmful agents [14]. This type of response requires a series of mechanisms that involve humoral factors, cell and tissue, antimicrobial peptides and complement factors. Humoral factors may be cellular receptors or molecules that are soluble in plasma and other body fluids [14].

5.2 Specific Immunity

The specific immune response occurs through mechanisms that involve a complex network of specialized cells, proteins, genes and biochemical messages that provide the means necessary for the body to respond specifically to antigens, antibodies and effector cells with high specificity and affinity [22].

6. THE POTENTIAL OF LEMNA SP AS FISH FEED

Lemna sp. (duckweed) is a type of aquatic plant that can be found growing in ponds, lakes, and reservoirs. The ability of this plant is to grow quickly and develop well in various climates. *Lemna* sp. become one of the aquatic plants that have not been utilized because it has no selling value and does not compete with human needs [23]. According to Solomon and Okomoda (2012), *Lemna* as a feed ingredient has better content than most other vegetable proteins and is more similar to animal protein. Protein content ranges from 10-45%, fiber 7-14%, carbohydrates 35%, fat 3-7%, and the content of vitamins and minerals is quite high. The protein content of dry duckweed is 25.2 – 36.5 % and the protein concentrate ranges from 37.5 – 44.7% [24].

Lemna sp. as a source of protein contributes to maintaining the availability of feed to support aquaculture activities. Fresh *lemna* as a source of feed from nature is very suitable to be used as feed for herbivorous fish. Herbivorous fish are fish that eat plants (vegetables). Herbivorous fish are generally easy to accept additional food or artificial feed. Some examples of herbivorous fish are tilapia, grass carp, milkfish, Nile tilapia, and tawes [25].

7. EFFECT OF LEMNA SP. FOR IMMUNITY RESPONSE

Lemna sp. is a small aquatic plant that has a high nutritional content. The ability of this plant is to grow quickly and develop well in various climates. *Lemna* sp. known as weeds in waters tend to be difficult to control growth. This aquatic plant is one of the aquatic plants that has not been utilized because it has no selling value and does not compete with human needs [23]. The ability of this easily cultivated aquatic plant is capable of producing secondary metabolites such as a wide variety of flavonoids including luteolin and apigenin which can be used as antioxidant compounds [26]. Flavonoids can function as antioxidants, immunomodulators, and anti-inflammatory agents, while several other substances are thought to increase fish immunity, namely vaccines, immunostimulants, probiotics, and antioxidants. The active compounds are mainly flavonoids, carotenoids, and amino acids of plant extracts including extracts from *Lemna* sp. able to play a role in stimulating leukocytes as a non-specific defense so that it functions as an immunostimulant [6].

Lemna sp. is a medicinal plant that does not have severe side effects. Recent studies have revealed various pharmacological effects of duckweed. *L. minor* has antibacterial activity against gram-negative bacilli (*Pseudomonas fluorescens*, *Shigella flexneri*, *Escherichia coli*, and *Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis*), and can be an alternative antibacterial for the treatment of various diseases. Flavonoids in *Lemna* sp. may contribute metabolites to antioxidant activity. In particular, the flavonoids in *L. minor* have been reported to have an immunosuppressive effect by reducing free hemoglobin levels and antibody production in human whole blood samples infected with ovalbumin antigen [12].

8. CONCLUSION

Flavonoids are antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic coupled with their capacity to modulate cellular enzyme function. Flavonoids also have the potential to stimulate the immune system. *Lemna* sp. is a medicinal plant that does not have serious side effects. The immune system in fish is known to consist of an innate

defense system or a non-specific innate/natural defense system and a specific immune system or adaptive response. *Lemna* sp. has immunomodulatory properties, namely the ability to increase phagocytosis so that it can increase the body's immunity in fish.

REFERENCES

1. Suwarsito., Mustafidah, H. Diagnosing Fish Disease Using Expert System. JUITA Vol. 4. 2011. Indonesia
2. Widianoro, W. Degrees of Survival and Health of Eel (*Anguilla bicolor*) in Two Different Growing Areas (Places). *Journal of Aquafish Saintek* 2020; 1(1): 35-38.
3. Buchmann, K. Evolution of innate immunity: clues from invertebrates via fish to mammals. *Frontiers in Immunology*, 2014; 23(5): 459.
4. Mulia D.S., Utomo, T., and Isnansetyo, A. The Efficacy of *Aeromonas hydrophila* GPI-04 Feed-based Vaccine on African Catfish (*Clarias gariepinus*). *Biodiversity*. 2022; 23(3). 1505-1510.
5. Armando, E., A. Lestiyani., R.A. Islamic. Potential Analysis of *Lemna* sp. Extract as Immunostimulant to Increase Non-Specific Immune Response of Tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *Research Journal of Life Science* 2021; 8(1).
6. Iskandar., D. Kurnia., Y. Mulyani., I. Zidni., A. Riyanto., Y. Andriani. Use of *Lemna* sp As Antioxidant in Feed and Its Effect on Nile Tilapia (*Oreochromis niloticus*) Performance. *ICONISTECH*. 2019. Indonesia.
7. Sugiyono. *Research Method*. 2003. Indonesian.
8. Panche, A. N., Diwan, A. D., Chandra, S. R. Flavonoids: an overview. *Journal of Nutrition Science* 2016; 5, e47, page 1 of 15
9. Jung, H.A., Jung, M.J., Kim, J.Y., Chung, H.Y., Choi, J.S. Inhibitory activity of flavonoids from *Prunus davidiana* and other flavonoids on total ROS and hydroxyl radical generation. *Arch. Pharm. Res.* 2003; 26, 809–815
10. Procházková, D., Bousová, I. & Wilhelmová, N. Antioxidant and Prooxidant Properties of Flavonoids. *Fitoterapia*, 2011; 82(4), pp. 513-523
11. Zarina, Z., Tan. S.Y. Determination of flavonoids in *Citrus grandis* (Pomelo) peels and their inhibition activity on lipid peroxidation in fish tissue. *International Food Research Journal* 2013; 20(1): 313-317
12. Hung, D.J., Ku, S.C., Tsan, C.C. and Chuan, L.H. Antioxidant potentials of buntan pumelo (*Citrus grandis* Osbeck) and its ethanolic and acetified fermentation products. *Food Chemistry* 2010; 118: 554- 558.
13. Rombout JH, Huttenhuis HBT, Picchiatti S, Scapigliati S. Phylogeny and ontogeny of fish leucocytes. *Fish and Shellfish Immunology* 2005; 19, 441–455.
14. Magnadóttir, B. Innate immunity of fish (overview). *Fish & Shellfish Immunology*. 2006; 20, 137-144
15. Kiron V. Fish immune system and its nutritional modulation for preventive health care. *JAnim Feed Sci Technol* 2012; 173: 111-133.
16. Mehana, E., Rahmani, A., & Aly, S. Immunostimulants and Fish Culture: An Overview. *Annual Research & Review in Biology*, 2015; 5(6), 477–489.
17. Van Muiswinkel, W. B., & Nakao, M. A short history of research on immunity to infectious diseases in fish. *Developmental & Comparative Immunology*, 2014; 43(2), 130–150.
18. Anderson, D. P. And Vaccine Carriers In Fish: Application to Aquaculture. *Annual Review of Fish Diseases*, 1992;(2):281–307.
19. Vadstein, O. The Use of Immunostimulation In Marine Larviculture: Possibilities and Challenges. *Aquaculture*, 1997;155(0044), 401–417.
20. Maqsood, Sajid, Singh, P., Samoon, M. H., & Balange, A. K. Effect of Dietary Chitosan on Non-specific Immune Response and Growth of *Cyprinus carpio* Challenged with *Aeromonas hydrophila*. *International Aquatic Research* 2010 Vol.2 No.2 Pp.77-85 Ref.29, 2(2), 77–85.

21. Whyte SK. The innate immune response of finfish e A review of current knowledge. *Fish and Shellfish Immunology* 2007; 23, 1127–1151.
22. Uribe, C., Folch, H., Enriquez, R., Moran, G. Innate and adaptive immunity in teleost fish: a review. *Veterinari Medicina*, 56, 2011 (10): 486–503
23. Asriyanti, I.N., J. Hutabarat., V.E. Herawati. Effect of Using Lemna Sp Flour. Fermented In Artificial Feed On The Level Of Feed Utilization, Growth And Survival Of Dumbo Catfish (*Clarias Gariepinus*) Seeds. *Journal of Aquaculture Engineering and Technology* Vol 7(1). 2018. Indonesia.
24. Solomon, S. G., Okomoda, V. T. Growth Performance of *Oreochromis Niloticus* Fed Duckweed (*Lemna minor*) Based Diets in Outdoor Hapas. *International Journal of Research in Fisheries and Aquaculture* 2012;2 (4): 61-65
25. Asriyana and Yuliana. *Aquatic Productivity*. Askara Earth. 2012. Indonesia.
26. Choi, S., Youn, J., Kim, K., Joo, D.H. WaveApigenin inhibits UVA-induced cytotoxicity in vitro and prevents signs of skin aging in vivo. *Int. J. Mol. Med.*, 2016;38(2):627– 634.

UNDER PEER REVIEW