

## Use of Nettle (*Urtica dioica L.*) in the Treatment of Fish Diseases

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### Abstract

The increase in demand for fish and fish products has led to the spread of the culture fish. Fish breeders have used some chemicals or plant extracts that stimulate or enhance the immune system to protect fish from diseases. Although the use of chemical medicines has many negative effects on the environment and human health, chemotherapy is widely used in the prevention and treatment of diseases. As an alternative to chemical treatments, the use of plant extracts in combating diseases seen in water products has become widespread. Various plant species such as thyme, garlic, fenugreek and nettle are used in the treatment of different diseases in traditional folk medicine as well as in fish health and increase disease resistance and efficiency in aquaculture.

Nettle is a perennial plant of the Urticaceae family, abundant in our country. Root, stem, leaves, flowers and seeds of nettle are used as herbal medicines. Nettle has many biocomponents such as polysaccharides, lignans, flavonoids, coumarins, terpenoids, phenol and *Urtica dioica* agglutinin (UDA) which is a lectin, and it has been reported that nettles generally have antiinflammatory, antiallergic, antibacterial, antifungal, antiviral and anticarcinogenic effects. There are studies in the literature that have found positive effects on the immune system and growth performance of nettle on experimental animals. In this review, a study has been conducted on the use of nettle weed as an alternative to chemical medicines in the prevention and treatment of fish diseases.

**Keywords:** Nettle, Fish Diseases, herbal medicine, immune system.

### INTRODUCTION

In recent years interest in natural antimicrobials has increased significantly in order to prevent fungal and bacterial deterioration. Antimicrobial substances can naturally be obtained from plants, animals, algae and fungi. Many chemicals derived from plants have been associated with antimicrobial activity and the effects of pathogens have been investigated [1]. In traditional folk medicine many plant and plant extracts have been used in the treatment of various diseases. In order to combat diseases from past to present day, plant therapy was applied [2].

Concerns about the safety of synthetic chemicals used against pathogenic microorganisms have increased and disease-causing microorganisms have gained resistance. For this reason, it is observed that the use of plant extracts for combating diseases is of interest for the researchers [3]. In addition, interest in herbal medicines has increased because of the many side effects of synthetic drugs and their cost. Treatment with herbal medicines now finds application in the livestock sector and is being used in such fields as treatment, increase of production, better quality and higher yield, preparation of feed rations [4].

The use of chemical medicines is prevalent in the fisheries sector, which is a major economic concern today, in order to prevent losses due to microbial diseases. However, the use of unconscious antibiotics and other medicines increases drug accumulation in fishes, as well as the resistance of microorganisms to antibiotics and their consumption has negative effects on the human immune system [5]. It is known that veterinary medicines have been widely used in aquaculture by participating in bathing, injection and eating for the prevention of economic losses and diseases due to various factors [6]. In Europe, amoxicillin, florfenicol, flumequine, oxolinic acid, oxytetracycline, sarafloxacin and sulfadiazineinethrimethoprim are antimicrobial agents permitted for use against external parasites and fungal diseases in aquaculture [7]. Unconscious drug use in this sector threatens the ecological balance as well as human health [8] and restrictions are imposed on veterinary drugs due to side effects [9].

### Use of Plant Extracts in Fish Diseases

Aquaculture has become very common in the world and our country in recent years. Along with growth in this sector, the widespread use of many medicines has been associated with the health of people, environment and animals. The continuous and widespread use of synthetic antimicrobials adversely affects consumers. Herbal medicines are applied safely against bacterial diseases in organic agriculture, veterinary medicine and medicine. Since ancient ages, many plants have been used against infectious diseases because of their antibacterial properties and they have made the plants an alternative natural medicine with potential use in aquaculture [10]. Parts of many plants such as seeds, leaves, bark, fruits and roots contain bioactive phenolic compounds [11] and these compounds exhibit antimicrobial properties [12]. Plants have a variety of bioactivity due to components such as alkaloids, terpenoids, tannins, saponins, flavonoids and essential oils [13]. Natural chemicals containing plants have advantages such as environment friendly, low cost treatment, easy biodegradation in nature, less drug resistance, no toxicity and no carcinogenic effects. It has been reported that plants or extracts such as garlic, ginger, olive and nettles have antipathogenic and similar properties, particularly in fish, that enhance appetite and growth performance, warn the immune system [6].

In fish, the immune system is activated by using chemical immunostimulants and body resistance is increased against infections. Like chemicals, medical plants also stimulate the mechanism of specific and non-specific immune system in fish. Plants increase body resistance and increase the level of readiness of the body against viral, bacterial and parasitic diseases [14], [15]. Researches on the use of herbal extracts in the prevention and treatment of aquaculture diseases are available in the literature, with a limited number of studies. For example, significant changes in the immune system and the number of erythrocytes, leukocyte count, hematocrit level, NBT level, total protein and total Ig parameters of fish have been determined as a result of application of black seed oil to rainbow trout in the form of rubbing, grafting and feeding. At the end of the 21<sup>st</sup> day, it was reported that despite

the decrease of the effect of the black seed oil, it strengthens the immune system and keeps the immune system at a high level if it is used regularly and at certain rates [16]. The use of plant extracts instead of chemical drugs against pathogenic microorganisms in fish is also available in the literature.

Antibacterial activity of vegetable oils obtained from cantonon, turmeric and sesame seeds against the pathogens isolated from fishes were investigated. In vitro studies have shown that seed oil can be used in the treatment of vibriosis and lactococcosis, showing antibacterial activity on *Vibrio anguillarum* and *Lactococcus garveiae* pathogens [17]. One of the plants with medicinal properties, walnut fruits, leaves and crusts have antifungal, antimicrobial, antidiarrhetic, hypotensive, hypoglycemic and antihelminthic effects [18]. It has been reported that extracts from walnut leaf and walnut green shells obtained from different walnut species (Lara, Franquette, Mayette, Marbot, Mellanaise and Parisienne) have antimicrobial effects against gram-positive bacterium (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) [19], [20]. The walnut shell and leaf extracts obtained from *Junglans regia* (British walnut), *Junglans cinere* (white walnut) and *Junglans nigra* (black walnut) were applied to freshwater fish. It has been shown to be effective against the common external pathogenic bacteria (*Aeromonas hydrophilia*, *Pseudomonas florescense*, *Bacillus*, *Salmonella*), fungus (*Ithyophorus hoferi*) and protozoans (*Chilodonella ciprinii*) [21]. In addition, methanol-extracted walnut leaf oil showed antimicrobial effects against pathogens (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Aspergillus niger*) isolated from skin, gill and digestive system tissues of fishes [5].

#### Nettle (*Urtica dioica L.*) and its Importance

Nettle is one of the plants used for the prevention of various diseases. This plant belongs to the family of *Urticaceae* [22] and is a perennial plant that grows spontaneously on rivers, roadsides and open forested areas of our country [23]. Nettle root, stem, leaves, flowers and seeds of nettle are used as herbal medicines. In the first century, Greek doctors Dioskorides and Galen reported that the nettle leaves were diuretic and laxative and they were used in the treatment of diseases such as asthma and lung inflammation. The nettle is used in folk medicine in almost every country [24]. It has been reported in the literature that nettle generally has antiinflammatory, antiallergic, antibacterial, antifungal, antiviral and anticarcinogenic effects [25]. Dry matter of stinging nettle leaf contains 18% protein, 14.5%-17% albuminous substances and 2.5% fatty substances. There are 8-10% fixed oil in the seeds and acetylcholine, histamine and formic acid in the burning hairs. Leaves contain K, vitamin B1, provitamin, urticin glycosides, cystosteine, xanthophylls, ash contains 6.3% ferrumtrioxide, silicon, potassium, calcium [26].

It contains a large number of bioactive compounds such as polysaccharides, lignans, flavonoids, coumarins, terpenoids, phenols and lectins [27], [28] and [29]. Because of its pharmacologically active metabolites, the leaves and seeds of the nettle (*Urtica dioica L.*) are used in the traditional folk medicine for the treatment of the disease such as eczema, abscess, wound healing, liver failure, rheumatic pain, internal diseases, diabetes, skin infections, nose bleeds, prostate and cancer [22].

#### Use of Nettle (*Urtica dioica L.*) in Fish Diseases

Combating diseases and obtaining high quality products in aquaculture is one of the main objectives. In many fish farms, various antibiotics, chemicals and substances that stimulate the immune system are used to fight against viral, bacterial, fungal and parasitic diseases [30]. The

immune system is important for fish to survive in a healthy way and resist infections. However, the chemicals used to develop the immune system are also responsible for the development of more resistant bacteria. In addition, the chemicals that accumulate in the tissues of fish also affect people who consume them. Commonly used antibiotics and sulphonamides are either destroyed early in the body or are less effective since they are not absorbed sufficiently. However, since plant extracts are organic molecules, their absorption in the body is easier and their activity is higher than the chemical ones [31]. There are few studies on the use of herbal medicines as a preventive or therapeutic agent in fish farming. However, interest in this area has increased in recent years due to the high cost of immune system stimulating chemicals used in aquaculture [31], [32].

There are studies in the literature on the positive effects of nettle (*Urtica dioica L.*) on immune system and growth performance on guinea pigs. Nettle (*Urtica dioica L.*) strengthens phagocytosis, cellular and humoral defense mechanisms against pathogens [13]. In 2015, a study conducted to investigate the haematological values of beluga fish (huso huso) fed with nettle-fortified fishmeal for 20-40-60 days. According to the control group, significant increases in lymphocyte, neutrophil and eosinophil counts were found on the 40<sup>th</sup> day (see Table-1). When the blood values of RBC, Hb, Hct, MCHC, MCH and MCV were also examined in the same study, it was found that the nettle increased these values at different feeding times according to the control group (see Table-2). [32].

**Table 1.** Differential Leukocyte Counts of Huso huso Fish after 40 Day Feeding [32].

Leukocyte Counts (%)	Lym (%)	Neu (%)	Eos (%)
Nette	95.00±0.0	8.00±0.0	2.00±0.0
Control	90.2±0.2	7.00±0.1	1.80±0.1

**Lym:** Lymphocyte, **Neu:** Neutrophil, **Eos:** Eosinophil

**Table 2.** Some Hematological Factors of Beluga Fish (huso huso) [32].

Day/ Blood values	RBC	Hb	Htc	MCHC	MCH	MCV
20 <sup>th</sup> day						
Nette	0.46	7.42	23.51	34.49	306.13	-
Control	0.42	3.8	20.53	19.09	186.26	-
40 <sup>th</sup> day						
Nette	0.81	7.26	-	40.47	90.43	-
Control	0.68	5.47	-	25.83	83.59	-
60 <sup>th</sup> day						
Nette	0.39	6.61	28.99	-	-	872.24
Control	0.36	5.81	17.57	-	-	753.69

**RBC:** Red Blood Cell, **Hb:** Hemoglobin, **Htc:** Hematocrit level., **MCHC:** Mean Corpuscular Hemoglobin Concentration, **MCH:** Mean Corpuscular Hemoglobin, **MCV:** Mean Corpuscular Volume

In 2013, another study conducted to determine the stimulating effects of different herbal extracts on immune system in fish. In the study, rainbow trout (*Oncorhynchus mykiss*) was fed with 1% and 0.1% nettle-fortified fish

meal for 3 weeks and the fish fed with nettle-fortified diet were found to have increased extracellular respiratory burst activity, intracellular activity, phagocytosis activity, blood leukocyte activity and plasma protein level compared to the control group (see Table-3). As a result, rainbow trout fed with bait containing nettle extracts reported that they stimulated the immune system against fish pathogens such as viruses, bacteria and fungi in the cellular and humoral defense mechanisms and could be used as an adjunct to the process of struggle against fish diseases of nettle [30].

**Table 3.** Non-specific Immune Activity of Rainbow Trout [30].

Plant (%)	Extra-cellular Burst activity (nmolO <sub>2</sub> /10 <sup>5</sup> leu)	Intracellular activity (NBT), (OD at 650 nm)	Phagocytosis (OD at 510 nm)	Plasma protein level (g/dl)
% 0.1 nettle	1.04	0.12	1.93	3.40
% 1 nettle	1.03	0.11	2.03	3.58
Control	1.02	0.10	1.88	2.48

*Aeromonas hydrophilia* is a pathogen that causes many diseases in the freshwater fishery, especially in the rainbow trout (*Oncorhynchus mykiss*) from blood poisoning to the ulcer. In 2009, *aeromonas hydrophilia* injected rainbow trout (*Oncorhynchus mykiss*) fed with fish meal containing of 1% (w/v) nettle for 14 days and the effect of nettle against this pathogen was investigated. Researchers found that nettle increased hematocrit and hemoglobin values, increased white blood cell (WBC) volume, monocyte, neutrophil counts and phagocytic activity compared to the control group. In addition, according to the control group, researchers reported that there was a decrease of 96% in

pathogen-injected fish mortality and that the nettle also decreased the number of bacterial colonies according to the control group (see Table-4). [33].

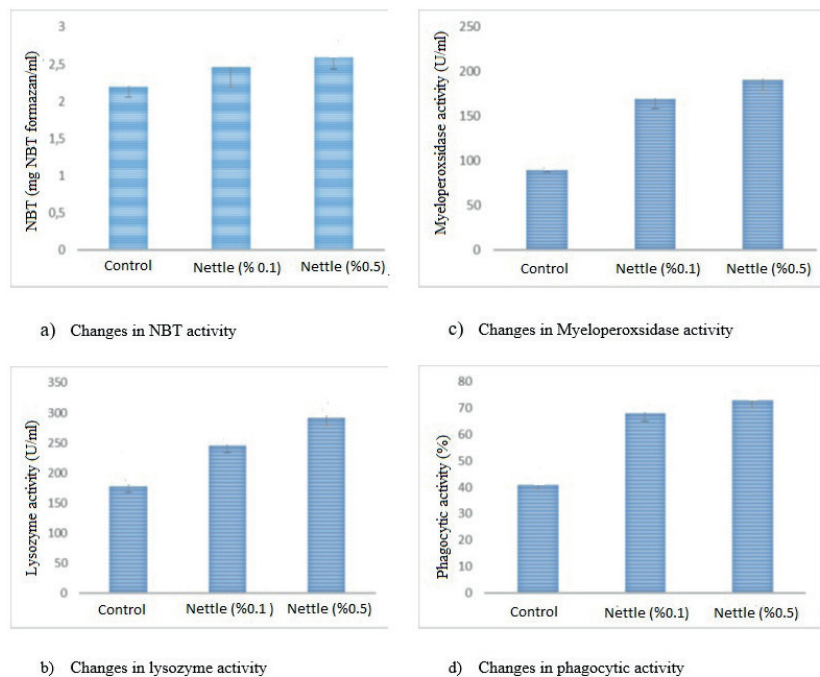
**Table 4.** Changes in RBC, WBC, Htc, Monocytes, Neutrophil values after feeding with nettle added fishmeal [33].

Plant (%)	RBCx10 <sup>6</sup> μL <sup>-1</sup>	WBC x10 <sup>4</sup> μL <sup>-1</sup>	Htc (%)	Monocytes	Neu
Control	1.09	3.5	31.8	4	3
%1 nettle	1.20	4.5	39.1	7	5

**RBC:** Red Blood Cell, **WBC:** White Blood Cell, **Htc:** Hematocrit level, **Neu:** Neutrophil.

The effects of the methanolic extract of the nettle on the immune system of Japanese fish (*Carassius auratus*) have also been examined. Two different concentrations of the nettle extract (0.1 and 0.5 g/kg bait) were incorporated into the fishmeal and the fishes were fed for 30 days. Superoxide radical release (NBT) is an important enzyme released in the inactivation of pathogens. Lysozyme activity is an immune response that disrupts the pathogen cell wall in viral, parasitic, and bacterial diseases. Myeloperoxidase (MPO) is an enzyme that is secreted by neutrophils and neutralizes foreign elements. Phagocytic activity indicates an increase in cell numbers that effect phagocytosis. For this reason, researchers used parameters such as superoxide anion production (NBT), lysozyme, myeloperoxidase and phagocytic activity in this study to determine changes in the immune system. At the end of the study, all immune responses were higher in both nettle groups than in the control group, and the highest immune response was found to be obtained with 0.5% nettle extract (see Table-5). Researchers have reported that nettle extracts are an effective immunostimulant for Japanese fish [34].

**Table 5.** Superoxide Anion Production (NBT), Lysozyme, Myeloperoxidase and Phagocytic Activity of Japanese Fish (*Carassius auratus*) [34].



Natural products containing rich antibacterial agents are important. Interest in natural antimicrobials with new and less toxic effects has increased. There are also studies in the literature investigating the use of nettle leaf extracts as an alternative to chemical drugs against fish pathogens. The most common fish pathogens such as *Aeromonas hydrophila* and *Yersinia ruckeri* which are gram-negative pathogens and *Streptococcus agalactiae*, *Lactococcus garvieae* and *Enterococcus faecalis* which are gram-positive pathogens, causes infectious diseases. *A. hydrophila* causes tail problems (tail/fin rot) and haemorrhagic septicemia. *Y. Ruckeri* which is an enteric pathogen causes redmouth disease in fish. Pathogens such as *S. agalactiae*, *L. garvieae* and *E. Streptococcus* cause lactococcosis, haemorrhagic septicemia and ulcerations in tails [10]. In

2012, nettle leaves extracted by using chloroform, n-hexane, methanol and ethyl acetate and their bioactive potential were investigated. Obtained extracts were applied in vitro to some common fish pathogens such as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Flavobacterium columnare*, *Vibrio salmonicida* and *Yersinia ruckeri*. In the study, it was determined that the highest inhibition zone (DIZ, mm) was in hexane-derived fractions. Gentamicin was used as standard and the antibacterial activities of the extracts were measured. Measured mean inhibition areas are given below (see Table-6). According to the results, researchers have been reported that the nettle leaf extract containing many bioactive components, so that it has potency as a natural drug in some infectious diseases seen in fish [35].

**Table 6.** Antibacterial Effect of Nettle Extracts Obtained Using Different Solvents on Some Fish Pathogens [35].

DIZ (mm)					
Fish pathogene / Solvent	<i>Aeromonas hydrophila</i>	<i>Aeromonas salmonicida</i>	<i>Flavobacterium columnare</i>	<i>Vibrio salmonicida</i>	<i>Yersinia ruckeri</i>
Hexane	12±0.4	12±0.4	11±0.5	13±0.5	14.66±0.8
Chloroform	7±0.3	7±0.3	-	7±0.3	-
Ethyl acetate	-	-	-	-	-
Methanol	-	-	-	-	-
Standart (Gentamycin)	17.66±0.3	17.66±0.3	18.33±0.3	20.66±0.6	20.66±0.6

## CONCLUSION

Fish are infected by pathogens and parasites such as viral infections, bacteria, fungi and protozoa. Medical plants have antimicrobial and immune system stimulating effects. When the literature is examined it is concluded that the extracts of many plant and plant parts such as canton, turmeric, garlic, sesame oil, blackcurrant, lemon, aloe vera, cinnamon, walnut and nettle are effective in prevention of pathogenic diseases encountered in aquaculture and especially fish farming. They have been used in the treatment and / or have potency of use. In this review, a study has been conducted on the use of nettle as an alternative to chemical medicines in the prevention and treatment of fish diseases. The presence of antimicrobial and antifungal properties of this plant has been associated with a terpene neoheptidene and fatty acid esters, heptadecyl ester, hexyl octyl ester, butyl tetradecyl ester and 1,2-benzenedicarboxylic acid [35]. It has been seen that the nettle leaves and root parts of the nettle are used in a limited number of antimicrobial applications in the literature. But if we summarize the work done, it is stated that nettle can be used as an alternative to veterinary medicines in the prevention and treatment of viral, bacterial and fungal diseases in fish studies. Nettle shows less toxicity because it is natural and provides an advantage in protecting the environment and human health. Nettle is a natural immunostimulant that enhances the immune system in fish and keeps defense mechanisms against pathogens ready. Due to the developing technology, increasing demand and decreasing natural resources have become a necessity of using drugs in the aquaculture. However, interest in organic products has increased due to possible side effects of chemicals. Nettle can also be used as an alternative to drugs used to increase yield in fish breeding. Unlike synthetic medicines that are used against possible infections, nettle can be preferred, which can be natural and easy to degrade. The use of herbal extracts in fish diseases makes the treatment cheap. Also, since herbal products and nettle contain many bioactive substances in the composition, pathogens have less resistance than synthetic drugs. Since it contributes to the prevention of the development of resistant microorganisms in this respect. Due to these advantages of the nettle, it is necessary to make use of it in the fishery sector and to carry out further researches.

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