



A Mini-Review: Effect of *Moringa oleifera* Leaves for Fish Health

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Disease attack is the biggest obstacle in fish farming activities and is very detrimental to cultivators. Disease control is an important factor to be recognized. The use of herbs is an alternative for disease control that is relatively safe. *Moringa oleifera* plant is known to contain several secondary metabolite compounds, namely alychalcone, flavonoids, tannins and steroids which have antibacterial and antioxidant properties. Several *M. oleifera* treatment studies are used to treat and prevent fish infected with disease. The purpose of this article is to describe the compounds contained in *M. oleifera* and their effect for fish health. *M. oleifera* has a positive effect on fish health, it can be used to treat and prevent disease in fish.

Keywords: Disease; *M. Oleifera*; immunostimulant; prevention; treatment.

1. INTRODUCTION

Disease is one of the obstacles in fish farming [1]. Disease attack not only causes slow fish growth, has an impact on mass fish mortality (80-

100%) [2] and is economically very detrimental to farmers. Diseases in fish that can cause high mortality are bacteria and viruses [3] The bacteria that often infect cultured fish are *Aeromonas hydrophila* [4], *Sreptococcus innae*

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[5], *Sreptococcus agalactiae* [6], *Pseudomonas anguilliseptica*, *Plesiomonas shigelloides*, *Micrococcus* sp. [7], *vibrio* sp. *Flavobacterium columnare* [8], *Mycobacterium fortuitum*, *Mycobacterium marinum*, *Edwardsiella tarda* [9]. Several viruses that can infect fish are viral hemorrhagic septicemia virus [10], infectious haematopoietic necrosis virus, koi herpesvirus, epizootic haematopoietic necrosis virus, nervous necrosis virus, and others [11]. Fish is a source of animal protein for humans, so the quality of the fish should be a concern for cultivators. The quality of fish can be define by free from diseases and harmful chemicals, so it is safe for consumption [12].

Antibiotics and vaccines have been widely used by farmers to treat fish diseases. The use of antibiotics for a long time can cause bacteria to become resistant, pollute the aquatic environment [13] and can accumulate in fish bodies [14], which of course will have an impact on reducing the quality of the fish [15,12]. Several studies have demonstrated the therapeutic effect of phage as a substitute for antibiotics in human and animal health problems [16-18].

Another obstacle is the limited number of vaccines that have been registered and marketed [19] Therefore, it is necessary to find and develop alternative materials as antibacterial and immunostimulant which are safer to fight disease.

M. oleifera is a medicinal plant from the Moringaceae family, known as Ben oil tree, horseradish tree, drumstick tree, benzolive tree, moringa, marango, mlonge, moonga. The plant comes from native to the sub-himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is now widely cultivated and naturalized in many locations in the tropics [20].

M. oleifera is reported to have many medicinal properties [21], among others, to treat diabetes, cancer, inflammation, improve heart blood circulation and as an antioxidant; antifungal as well as antibacterial [22]. According to [23] the leaves part by pounding can be used as an itching medicine, clean the blood and prevent cancer. The flowers can be used as a tonic, diuretic, arthritis pain, and eye wash, the shoots part are used for liver, kidney, and joint pain, the root is used for bloating and fever. The roots are dissolved and applied to the skin to treat skin

irritation. The seeds are used to treat fever, rheumatism, and skin pain. The leaves can be used as antioxidants [24]. As a traditional medicine, *M. oleifera* is used to treat stomach diseases, cholesterol, gout, diabetes, high blood pressure [25]. Several studies have shown that the compounds contained in *M. oleifera* act as antibacterial and antioxidant [26, 27] to treat and increase fish resistance to disease [28, 29]. This article aims to describe the compounds contained in *M. oleifera* leaves and their role in fish health.

2. PHYTOCHEMICAL SCREENING of *Moringa oleifera* LEAVES

M. oleifera originating from South Asia and now found throughout the tropics [30], including the family Moringaceae and order Brassicales. This medicinal plant reaches 10 m in height, branched and the stem is brittle [31].

Secondary metabolite compounds contained in plants function for their own protection, besides that they can be used for human and animal health. Secondary metabolites are divided into three main components, namely polyphenols which include flavonoids and phenols, terpenoids, and alkaloids [32]. Flavonoids are found in all parts of higher plants including leaves, roots, wood, bark, pollen, flowers, fruits and seeds to various degrees [33]. Leaf parts are widely studied regarding phytochemical content, antibacterial testing, treatment and prevention of disease [34,35].

The results of phytochemical screening with various solvents showed that the secondary metabolites contained in *M. oleifera* leaves were alkaloids, flavonoids, tannins, saponins, phenols and terpenoids. Steroids were not detected with ethanol, ethyl acetat and N hexane as solvents, due to different types of solvents. Phenol is detected with ethanol solvent. The ethahol solvent was better than the ethyl acetate and N-hexan solvents (Table 1). Tannins in *M. oleifera* leaves extract were detected with all solvents, this is because quantitatively the tannin content was higher than the other components, namely 2.85% [36], whereas according to [37] it was 8.22% and 0.19% phenol content, alkaloids 0.42% and saponins 1.75%. In the ethanol extract of the leaves of *M. oleifera* contains achaloids 2.90%, 4.00% flavonoids, 3% tannins and 2.25% phenols [38].

Table 1. Phytochemical screening of *Moringa oleifera*

Solvents extract	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Phenol	Terpenoids	Ref
Ethanol	+	+	+	-	-	+	-	38
	+	-	+	+	-	+	-	37
	+	+	+	+	-	-	+	39
Methanol	+	+	+	+	-	-	-	40
Ethyl acetat	+	-	+	-	-	-	-	32
N-hexan	+	-	+	-	-	-	-	32
Methanol	+	+	+	+	+	-	-	41
Chloroform	+	+	+	+	-	-	-	41

Table 2. Antibacterial activity of *M. oleifera*

Extract solvents	Concentration extract	Type of bacteria	Inhibition zone (mm)	Ref.
Ethanol	10%	<i>Pseudomonas aeruginosa</i>	19,60	39
Ethanol	(200µ L/disc)	<i>Staphylococcus aureus.</i>	28	42
Aqueous	(200µ L/disc)	<i>Staphylococcus aureus</i>	25	38
Ethanolic	0,2 g/mL	<i>Streptococcus sp.</i>	9	38
Aqueous	0,2 g/mL	<i>Streptococcus sp.</i>	4	38
Petroleum ether	0,2 g/mL	<i>Streptococcus sp.</i>	7	38
Ethanolic	0,2 g/mL	<i>Staphylococcus aureus.</i>	8	38
Aqueous	0,2 g/mL	<i>Staphylococcus aureus.</i>	4	38
Petroleum ether	0,2 g/mL	<i>Staphylococcus aureus.</i>	12	38
Ethanolic	10 g/190 mL (100µ L/disc)	<i>Staphylococcus aureus.</i>	17,3	47
Aqueous	10 g/190 mL (100µ L/disc)	<i>Staphylococcus aureus.</i>	15,2	47
Ethanol	-	<i>Staphylococcus aureus.</i>	9	37
Ethyl acetat	-	<i>Staphylococcus aureus.</i>	10	37
Ethanol	-	<i>E. coli</i>	4	37
Ethyl acetat	-	<i>E. coli</i>	8	37
Ethanol	-	<i>Bacillus sp.</i>	18	53
Ethanol	100%	<i>Aeromonas hydrophila</i>	9.90 ± 1.62	54
Chloramphenicol	20 µg/mL	<i>E.coli</i>	30	43
		<i>S.typhi</i>	25	
		<i>Pseudomonas aeruginosa</i>	20	
Aqueous	20 µg/mL	<i>E.coli</i>	20	43
		<i>S.typhi</i>	18	
		<i>Pseudomonas aeruginosa</i>	-	

3. ANTIBACTERIAL ACTIVITY

Bacterial disease is an obstacle for fish cultivators in the world. The search and development of ingredients as safe antibacterials that do not cause side effects is needed to be

applied. This prompted investigations of plant-sourced antimicrobials. *M. oleifera* has been used as the object of research, because it has several benefits, especially its ability as an antibacterial [39]. *M. oleifera* potential antimicrobial activity against gram-positive and

gram-negative bacteria, and fungi [40]. Several studies have proven that *M. oleifera* leaf extract is antibacterial for pathogenic bacteria that infect cultured fish, namely *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Streptococcus* sp., *Staphylococcus aureus*. The sensitivity effect of *M. oleifera* as antibacterial depends on the type of solvent used in the extract preparation. Methanol extracts of *M. oleifera* inhibit bacteria better than ethanol extracts [41]. Effectiveness of ethanolic extracts of *M. oleifera* against both gram positive and negative bacteria [42]. Moringa leaf ethanol extract at a concentration of $\geq 4\%$ has antibacterial activity against *P. aeruginosa* [43]. Extract of *M. oleifera* at a concentration of 0.6 g mL^{-1} with petroleum ether solvent produced the greatest zone of inhibition against *Streptococcus* sp. compared with ethanolic and aqueous (water) solvents, 12 mm, 8 and 7 mm respectively [38]. Aqueous extract of *M. oleifera* has the least antimicrobial effect on *Streptococcus* sp. Aqueous extracts of plants generally exhibited little or no antimicrobial activity [44]. The ethanolic extract of *M. oleifera* at a concentration of $10 \text{ g} / 190 \text{ mL}$ ($100 \mu \text{L} / \text{disc}$) produced a larger inhibition zone diameter compared to Aqueous extract against *Staphylococcus aureus* bacteria, 17.3 mm and 15.2 mm, respectively [45] (Table 2). The antibacterial mechanism of secondary metabolites contained in *M. oleifera* leaves, namely flavonoids by inhibiting nucleic acid synthesis, damaging the function of the cytoplasmic membrane, inhibiting bacterial metabolism and synthesis of bacterial cell membranes and aggregating bacterial cells [46]. Tannins are phenolic compounds that inhibit bacterial growth by binding and precipitating protein [47], inhibiting extracellular enzymes and bacterial metabolism by inhibiting bacterial oxidative phosphorylation reactions [48]. The mechanism of saponin in inhibiting bacterial growth by reducing surface tension, resulting in increased permeability or cell leakage and resulting in intracellular compounds to come out [49]. Alkaloid compounds in inhibiting bacterial growth are by disrupting the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not formed completely and causes cell death [50]

4. TREATMENT EFFECT OF *M. oleifera* LEAVES EXTRACT ON FISH

Research has proven that *M. oleifera* leaves are used for the treatment of diseases in fish infected with pathogenic bacteria. *Clarias gariepinus*

infected with *Aeromonas hydrophila* experienced healing after being treated with *M. oleifera* leaf extract at 50% concentration through feed ration without adverse effect, indicated by increased levels of erythrocytes that support increased blood oxygen and increased leukocytes which function to suppress infection due to these bacterial attacks. Fish erythrocyte levels at the time of infection were $1.90 \pm 0.6 (\times 10^{12} \text{ cells} / \text{mm}^3)$ after treatment was $2.8 \pm 0.9 (\times 10^{12} \text{ cells} / \text{mm}^3)$, while fish leukocyte levels at the time of infection were $610 \pm 334.8 (\times 10^7 \text{ cells} / \text{mm}^3)$ and after treatment was $700 \pm 442.7 (\times 10^7 \text{ cells} / \text{mm}^3)$ [51].

The results of research conducted by [52] ethanol extract of *Moringa oleifera* leaves can treat *Carassius auratus auratus* fish infected with *Argulus* sp through immersion for 12 hours. A concentration of $62.5 \text{ mg} / \text{l}$ resulted in the highest reduction in infestation, which was 30%.

A study was conducted to investigate the effects of aqueous leaves extract of *Moringa oleifera* in the histology of the skin and gill of *Clarias gariepinus* challenged with *Ichthyophthirius multifiliis*. Six concentrations of aqueous leaves extract of *Moringa oleifera* were exposed to ich-infested fish for 1 hour to limit the impact of the adult parasite (trophont) in juveniles of *Clarias gariepinus*. The results showed that *Ich* infestation on the skin and gills was significantly reduced compared to negative controls ($p < 0.05$). Damage to the skin and gill organs in the form of healing lesions, edema ($p = 0.041$), severe damage to secondary lamellae ($p = 0.025$), fatty degeneration ($p = 0.041$) and inflammatory infiltrates ($p = 0.02$) [53]

5. EFFECT OF *M. oleifera* LEAVES EXTRACT AS AN IMMUNOSTIMULANT

Research on *M. oleifera* as an alternative to prevent disease has been widely carried out, because *M. oleifera* contains flavonoids and saponins which have immunostimulant effects that can increase non-specific resistance to disease. Apart from that these compounds, it acts as an antioxidant that can stop free radical chain reactions [54]. The results showed that the index antioxidant activities of *M. oleifera* by IC_{50} ($248.85 \mu \text{g} / \text{mL}$) was categorized as medium scale [55]. The level of antioxidant power is divided into 4 levels, namely very strong ($\text{IC}_{50} < 50 \mu \text{g} / \text{mL}$), strong ($\text{IC}_{50} 50\text{-}100 \mu \text{g} / \text{mL}$), moderate ($\text{IC}_{50} 100\text{-}250 \mu \text{g} / \text{mL}$, and weak ($\text{IC}_{50} 250\text{-}500 \mu \text{g} / \text{mL}$) [56]. The results of the

study [57], the antioxidant activity of *M. oleifera* extract were evaluated by hydroxyl radical scavenging activity, showing that the *M. oleifera* extract with methanol and chloroform solvents showed the same activity with different concentrations of 150 and 250 µg-ml, respectively. The extract concentrations were observed under UV-visible spectroscopy at 412 nm and the results were 0.284 and 0.365 nm. This shows that *M. oleifera* leaves have antioxidant activity, due to the presence of polyphenolic compounds such as flavonoids which can donate hydrogen atoms to OH radicals, thereby inhibiting the oxidation process [58]. Saponins function as immunostimulating agents [59]. The results of experiments conducted by [60] showed that saponins have an effect on the immune system of animals.

The function of immunostimulants is to induce macrophages to produce interleukins which will make lymphocytes divide into T-lymphocytes and B-lymphocytes and make B lymphocytes more active in producing antibodies [61]. Increased body immunity is characterized by an increase in the number of leukocyte. As according to [62] the increase in leukocyte cells is a reflection of the success of the fish immune system in developing a (non-specific) cellular immune response as a trigger for the immune response.

(*Oreochromis niloticus* Linn) fry after being given *M. oleifera* extract at doses of 50, 75 and 100 mg through intra-muscular injection, there was an increase in the number of leukocyte cell. The increase in the amount of leukocyte cell at doses of 75 and 100 mg was quite high, 187.09% and 148.56%, respectively, but the highest survival occurred at a dose of 75 mg (83.33% ± 5.77) with a relative percent survival value (RPS) of 72.22% ± 9.62 [63]. The normal range for RPS is > 60% [64].

M. oleifera leaf extract 153.95 kg⁻¹ of feed was effective in inducing resistance to sangkuriang catfish (*Clarias gariepinus*) fry, with an increase in leukocyte cell by 23.16 ± 6.46%. For 14 days after being challenged with *Aeromonas hydrophila* catfish seeds did not show clinical symptoms of bacterial infection, the response to feed and shock was also quite good and resulted in the highest survival, which was 80.0 ± 5% [28]. The increase in the number of leukocyte cells in catfish is a response of fish to increase their body resistance as defense cells [29].

6. THE ANTRINUTRIENT EFFECT OF *Moringa oleifera* ON FISH HEALTH

The anti-nutritional substances contained in *M. oleifera* leaves are tannins and saponins [65]. Tannin can bind to protein and amino acids, as well as bind to other macromolecular compounds such as carbohydrates, especially starch and cellulose, minerals Ca, P, Fe and Mg, as well as vitamin B12. Tannins in the digestive tract can cover the mucosal walls of the digestive tract causing reduced absorption of nutrients [66]. Saponins decrease the permeability of small intestinal mucosal cells, which results in inhibition of active nutrient transport and causes the uptake or absorption of nutrients in the digestive tract to be disturbed. Saponins have an influence on the body's biological processes and the metabolism of nutrients by inhibiting the productivity of enzymes such as chemotrypsin enzymes, thereby inhibiting livestock productivity and growth [67].

According to [68] the antinutrient substances in *M. oleifera* are generally low and if consumed by humans or animals does not have a negative impact on health. The saponins contained in *M. oleifera*, although they taste bitter, do not have any harmful effects [69]. However, the lower the amount of antinutrients the better [70]. The saponins contained in *M. oleifera*, although they taste bitter, do not have any harmful effects [71]. However, the lower the amount of antinutrients the better [72].

7. CONCLUSION

M. oleifera gives a positive effect on health of fish, which can be used to treat and prevent fish diseases.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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