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Potential Bioactive Compounds of *Melastoma malabathricum* Leaf Extract in Feed on Growth Performance and Survival Rate of Tilapia Fry in Brackish Water

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Abstract

Tilapia have a salinity tolerance of 0 to 10 ppt. However, the increase in salinity causes a decrease in the growth rate of these fish. In addition, the rapid maturation of the gonads causes this fish to experience a slowdown in growth. This study aimed to know the effect of giving *Melastoma malabathricum* extract on the growth performance of tilapia fry reared in brackish water. The study consisted of treatment with 0, 1, 3, and 6 g/kg of feed with three replications of *M. malabathricum* leaf extract. *M. malabathricum* leaf extract was mixed in feed with a CMC concentration of 2%. Feed is given as much as 3% body weight twice a day. Observations of growth performance were carried out every seven days, and the amount of feed was readjusted based on the results of sampling fish weight. The study was conducted for 70 days using brackish water with a salinity of 8 ppt. Based on the study results, it was found that the highest growth of tilapia fry at the end of the study was found in the treatment of 1 g/kg feed. The same thing was also found in measuring feed efficiency, survival, and specific growth rate. It indicated that the best concentration of *M. malabathricum* leaf extract in supporting the growth performance of tilapia fry was 1 g/kg feed.

INTRODUCTION

Tilapia (*Oreochromis niloticus* L.) is one of the popular fish commodities to be cultivated and has a wide salinity tolerance, where the optimum salinity of tilapia fry is 0 to 10 ppt. The advantage of tilapia is that it can be cultivated in ponds using brackish water. However, the slow growth of these fish in brackish water is still a problem. In addition, the main problem faced by cultivators is the high mortality rate at the beginning of stocking. Cultivators commonly use efforts to suppress mortality when stocking fish seeds by using chemical drugs that directly reduce the quality of fish and pond water.

The approach used in overcoming the problem of low seed quality is to improve feed quality. It can be done by adding ingredients that have the potential to increase survival and stimulate the increased growth performance of these fish

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seeds. The utilization of plant extracts is one solution to suppressing the use of drugs, chemical compounds, and hormones in the fish farming process because they have low toxicity. Especially for tilapia, the primary use of plant extracts is to suppress reproduction during the cultivation process. The development of research related to *M. malabathricum* leaves has previously been studied by freshwater tilapia (Samalei *et al.*, 2021). The object of its research is gonadal development and growth. Based on the study results, it was known that this leaf extract at a specific dose could inhibit the development of good gonads in freshwater tilapia.

The potential for tilapia cultivation in brackish waters is wide, considering that tilapia has a wide salinity tolerance, in addition to its relatively fast growth and does not require special handling (Washim *et al*., 2022). The advantage of tilapia is that it can be cultivated in ponds using brackish water. However, the slow growth of these fish in ponds is still a problem. Farizah *et al*. (2017) reported that the leaf extract of *M. malabathricum* contains bioactive compounds of flavonoids, enols, saponins, steroids, tannins, and triterpenoids, in which some of these bioactive compounds have anti-fertilization properties. Information regarding the effect of *M. malabathricum* leaf extract on growth in saline tilapia fry is not yet available. Therefore, it is essential to conduct this study to know the effect of giving *M. malabathricum* extract on the growth performance of saline tilapia fry reared in brackish water.

METHODOLOGY

Place and Time

This research was conducted in the indoor room of the Tilapia division, Brackish water Aquaculture Development Center Takalar, Ds. Bontoloe Kec. Galesong Kab. Takalar, South Sulawesi, Indonesia, and the Water Productivity and Quality Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University Makassar, South Sulawesi, Indonesia, from December 2021 to February 2022.

Research Materials

The materials used in this research were tilapia fry with an average weight of 0.8 g/fish obtained from the second derivative of the process (F2) salinity adaptation of Tilapia strain Nirwana from the Brackishwater Aquaculture Development Center Takalar, South Sulawesi. The feed used was commercial feed (fish larvae feed MS Prima Feed PF 0).

The tools used in this study were fish reared media volume of 20 liters, aerator, digital balance, dark glass container, filter paper, rotary vacuum evaporator, and freeze dryer.

Research Design

A completely Randomized Design (CRD) with three treatments and three replications was used in this research (Figure 1) with the following composition: A (without enrichment of *M. malabatricum* extract in feed or control), B (*M. malabatricum* extract as much as 1 g per kg of feed), C (*M. malabatricum* extract as much as 2 g per kg of feed), D (*M. malabatricum* extract as much as 3 g per kg of feed). Analysis of feed proximate enriched with different doses of *M. malabathricum* leaf extract was conducted based on the AOAC method (1990), as shown in Table 1.

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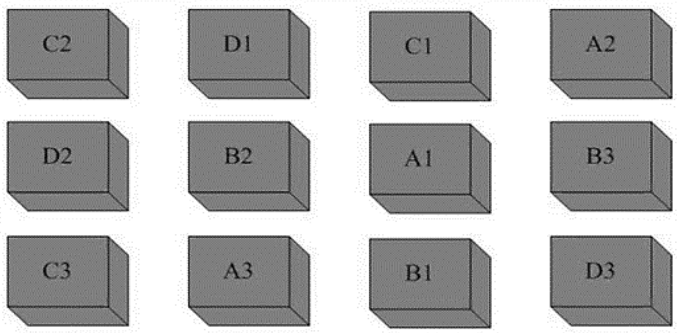

Figure 1. The layout of the research container.

Table 1. Proximate composition of fish feed with different dried *M. malabathricum* leaf extracts used for four experimental trials.

Parameters	*M. malabathricum* leaf extract in fish feed (g/kg)			
	A (0) (control)	B (1)	C (3)	D (6)
Moisture (%)	10	12.4	12.63	11.53
Ash (%)	12	10.66	10.75	10.69
Crude lipid (%)	6	6.45	6.91	7.5
Crude protein (%)	42	42	42	42
Crude fiber (%)	2	2.06	2.02	1.81

Work Procedure

M. malabathricum Leaf Extraction

The process of extracting the leaves of *M. malabathricum* was carried out based on Farizah *et al*. (2017). *M. malabathricum* leaves were extracted using ethanol by maceration using 80% ethanol as solvent. 500 g of leaf powder was put into a dark glass container with a lid, macerated with ethanol solvent with a concentration of 80%, covered, left at room temperature and protected from light for 3 x 24 hours, stirring frequently. Then the maceration solution was filtered with filtrate paper, and the dregs were macerated again using the same procedure. The work was repeated three times. The obtained macerates were combined and then evaporated using a rotary vacuum evaporator at a temperature of ±40 °C until a thick extract was obtained. Then the thick extract is made into flour through freeze-drying.

Feeding Treatment Enriched with *M. malabathricum* Extract

They were fed twice as much as 3% body weight (morning and evening). A daily meal was readjusted every seventh day. The feed used is commercial feed (fish larvae feed MS Prima Feed PF 0) in the form of flour. Extraction of *M. malabathricum* leaves was dissolved in CMC (carboxymethyl cellulose) solution with a concentration of 2% (Sari *et al.*, 2016) and then air-dried. A total of 20 fish per treatment with each treatment and replication. Fish were kept in containers with a 2 fish/liter density, and water salinity was maintained at 8 ppt. Maintenance was carried out for 70 days.

Growth Parameters

Observation of fish growth was carried out weekly by weighing the weight of the fish. Indices for determining growth performance were evaluated by the following; The test parameters carried out in this study were Weight Gain (Wm), Feed Efficiency (FE), Survival (SR), and Specific Growth Rate (SGR).

The growth of fish body weight was measured using the following formula:

$Wm = Wt - Wo$

Where:

Wt = final weight (g)

Wo = initial weight (g)

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Fish feed efficiency is measured using the formula:

$$FE = \frac{(W_t + D) - W_0}{F} \times 100\%$$

Where:
Wt = average weight of fish at time t (g)
Wo = average weight of fish at the beginning (g)
D = dead fish weight during rearing (g)
F = amount of feed given (g)

Fish survival was measured using the formula:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where:
SR = survival rate (%)
Nt = number of fish at time t (individual)
No = number of fish at the beginning of the experiment (individual)

The specific growth rate of fish was measured using the formula:

$$SGR = \frac{LnW_t - LnW_0}{t} \times 100\%$$

Where:
SGR = specific growth rate
Wt = weight of fish at the end of the study
Wo = weight of fish at the beginning of the study
t = research time (length of study)

Data Analysis

The data obtained were analyzed using the ANOVA statistical test and the Tukey test as a further test to see the real difference and analyzed descriptively using tables and graphs.

RESULTS AND DISCUSSION

Growth

Based on the results of the study, it was known that the growth trend of saline tilapia seeds was not significantly different ($P<0.05$) until the 42nd day of observation (Figure 2). It is thought to be because the compounds contained in *M. malabathricum* leaf extract require time in the absorption process to give different effects from each treatment on the growth of saline tilapia fry. The difference in growth trend from day 49 showed that the treatment of 1 g/kg of feed enriched with *M. malabathricum* leaf extract was significantly different ($p<0.05$) from other treatments until the end of the observation. It shows that enrichment of 1 g extract per kg of feed gave the best results in stimulating the growth of saline tilapia fry.

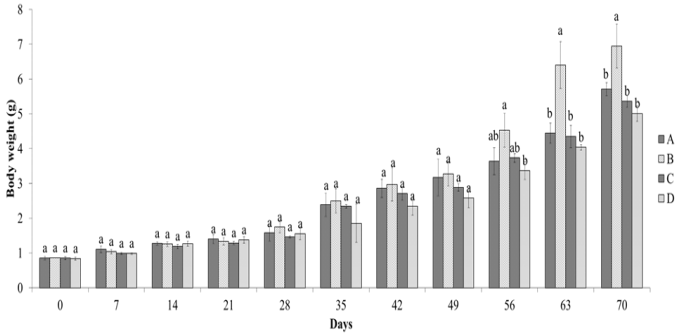

Figure 2. Daily weight gain of saline tilapia fry per observation time.

Table 2. Weight gain of saline tilapia fry.

Treatment	Weight gain (g)
	Average ± SD
0 g / kg of feed (control)	4,857 ± 0,140^{b}
1 g / kg of feed	6,090 ± 0, 632^{a}
3 g / kg feed	4,507 ± 0,206^{b}
6 g / kg feed	4,167 ± 0,261^{b}

Note: Numbers followed by different superscript letters in the same column are significantly different based on the Tukey test ($P<0.05$).

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The highest average weight gain was found in the treatment of feed enriched with 1 g of *M. malabathricum* leaf extract per kg of feed (Table 2). Based on statistical analysis, treatments significantly affected weight gain with a 95% confidence level. It indicated that the administration of *M. malabathricum* leaf extract affected the growth of saline tilapia fry. Further analysis using the Tukey method showed a significant difference between the treatments, where the 1g/kg feed treatment was significantly different from the other treatments and the control ($P<0.05$). The same thing was also reported by Samalei *et al*. (2021), where the best concentration in the average weight gain of tilapia fish reared in freshwater was 1 g/kg, and the results were significantly different from the other treatments ($P<0.05$).

The benefits of herbals in stimulating fish growth have been widely reported (Ahmadifar *et al.*, 2021). According to Wang *et al*. (2017), the growth rate of Japanese seabass given additional feed in the form of Chinese Herbals medicine mixture (CHMM) is associated with increased digestion and nutrition absorption. A more in-depth study was conducted by Midhun *et al.* (2016), where it is known that the addition of curcumin in feed was able to increase the activity of digestive enzymes and also modulate the expression of growth hormone (GH) and growth factors such as insulin-like growth factor 1 (IGF-1) and IGF-2 in *Oreochromis mossambicus* meat. According to Ahmadifar *et al*. (2021), growth hormone (GH) and IGF-1 in fish play an essential role in regulating growth. It is believed to be influenced by several factors, such as the organism's environmental, genetic, and nutrition (Triantaphyllopoulos *et al*., 2020).

Feed Efficiency

Feed efficiency (FE) is the ratio between the weight gain of fish and the total weight of feed consumed during rearing in percent. The greater the percentage value obtained, the more efficient it is in using feed for fish growth. Increasing FE is essential to lowering production costs in aquaculture and attaining long-term sustainability for the aquaculture industry as a whole (de Verdal *et al*., 2018). Based on the results of the calculations in Table 3, it is known that 1 g of extract per kilogram of feed results in maximum feed efficiency. This demonstrates that the administration of *M. malabatricum* leaf extract has a beneficial effect on the feed efficiency of fish.

Table 3. The efficiency of feed enriched with *M. malabathricum* leaf extract on saline tilapia fry.

Treatment	Feed efficiency (%)
	Average ± SD
0 g / kg of feed (control)	59,052 ± 2,570[a]
1 g / kg of feed	65,052 ± 1,944[a]
3 g / kg feed	56,871 ± 3,295[a]
6 g / kg feed	57,256 ± 5,965[a]

Note: Numbers followed by the same superscript letter in the same column are not significantly different based on the Tukey test ($P>0.05$).

The 1 g/kg concentration of *M. malabathricum* leaf extract had the highest feed efficiency, but the Tukey test ($P > 0.05$) revealed that it was not substantially different from other treatments (Table 3). The feed efficiency number corresponded to the findings of growth calculations, where the best results were obtained by adding 1g/kg of *M. malabathricum* leaf extract to the feed. According to Kanis and Koops (1990), the relationship between daily weight gain and feed efficiency is substantial.

The treatment of *M. malabathricum* leaf extract in the feed significantly affected the increase in feed efficiency,

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which positively affected the growth of tilapia seeds. An increase in FE was thought to be due to the presence of bioactive compounds from the melastoma herbal plant. Melastoma leaves contain steroids (sitosterol), triterpenoids (squalene), flavonoids, saponins, phenols, and α-tocopherol (Farizah *et al*., 2022). The content of these bioactive compounds plays a role in increasing the appetite of fish. One of the bioactive compounds that play a role in flavonoids and tannins, which increase fish's immune system and appetite (Mutrikah *et al*., 2018). Melastoma is included in herbal plants, where herbs can increase the body's resistance to disease, improve the digestive system, save on feed use, and increase fish appetite (Citarasu, 2010).

Survival Rate

The survival rate is the percentage of the number of fish that live in a particular time (Effendie, 1997). Based on the study results, it was known that the highest saline tilapia fry survival was obtained in the treatment of 1 g of *M. malabathricum* leaf extract per kg of feed. Based on the results of the Tukey test, it was found that each treatment was not significantly different (P>0.05) (Table 4). The lowest value came from the treatment of 6 g/kg feed. The high concentration of bioactive compounds in the leaf extract of *M. malabathricum* in the feed caused the survival of tilapia fry in brackish water to decrease. It may be due to the excessive dose of *M. malabathricum* leaf extract in the feed, causing negative feedback on the immune system of saline tilapia fry.

Table 4. Survival rate of saline tilapia fry.

Treatment	Survival Rate (%)
	Average ± SD
0 g / kg of feed (control)	83,333 ± 5,000[a]
1 g / kg of feed	95,000 ± 5,773[a]
3 g / kg feed	83,333 ± 15,275[a]
6 g / kg feed	80,000 ± 10,000[a]

Note: Numbers followed by the same superscript letter in the same column are not significantly different based on the Tukey test (P>0.05).

The highest survival rate was obtained from tilapia seeds fed with 1 g/kg feed containing *M. malabathricum* leaf extract. It is thought to be because saponins, one of the bioactive compounds contained in this extract, can increase the natural immunity of tilapia seeds so that their survival ability is better than other treatments. According to Ahmadifar *et al*. (2021), plants' bioactive compounds can act as immunostimulants and affect several immune-related pathways. Furthermore, Paray *et al.* (2020) explained that secondary metabolites, including saponins, have a high potential in treating infections.

Specific Growth Rate

The specific growth rate is a calculation of the daily growth of fish (Sulastika *et al.,* 2019). Based on the study results, it was found that the highest percentage of the specific growth rate of tilapia fry was found in the extract enrichment treatment of 1 g/kg of feed (Table 7).

The lowest value was found in the control treatment. Based on the results of statistical tests, it was known that the administration of *M. malabathricum* leaf extract had a significant effect on the specific growth rate with a 95% confidence level. Based on the results of the Tukey test, it was found that the enrichment treatment of extract 1 g/kg of feed was significantly different from the control. However, treatments of 1, 3, and 6 g/kg of feed were not significantly different.

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Table 5. The specific growth rate of saline tilapia fry.

Treatment	Specific Growth Rate (%/day)
	Average ± SD
0 g / kg of feed (control)	2,717 ± 0,037[b]
1 g / kg of feed	2,980 ± 0,133[a]
3 g / kg feed	2,625 ± 0,104[ab]
6 g / kg feed	2,550 ± 0,139[b]

Note: Numbers followed by different superscript letters in the same column are significantly different based on the Tukey test (P<0.05).

Based on this study, it was known that the highest growth, weight gain, and specific growth rate in the enrichment treatment of *M. malabathricum* leaf extract was 1 g per kg of feed. It was probably due to the bioactive compounds contained in this leaf extract working to inhibit the development of gonads in the early growth period of this fish. However, this related research should be done further. The bioactive compound contained in the leaf extract of *M. malabathricum*, which plays a role in inhibiting gonadal development, is called sitosterol (Farizah *et al.,* 2017). Previous researchers have proved that the leaf extract of *M. malabathricum* given to female tilapia broodstock and continued by giving the extract to the resulting seeds was able to significantly increase the percentage of male seeds (p<0.05) as the concentration increased. This leaf extract is applied to the resulting fry, up to 4 g per kg of feed kept in freshwater fish (Samalei, *et al.,* 2021).

In this study, the measurement results of the growth parameters showed that the higher the concentration of the leaf extract of *M. malabathricum*, the slower the growth. It may be due to the excessive content of bioactive compounds in the leaves of *M. malabathricum* in the feed, causing negative feedback on the fish. According to Farizah *et al*. (2017), *M. malabathricum* leaf extract contains several bioactive compounds, one of which is saponins. Saponins have antinutritional properties. Tian *et al.* (2018) reported that soybean saponins at concentrations of 5 and 10 g/kg feed significantly reduced growth performance and feed efficiency and damaged the morphology of the intestinal mucosa. It was further explained that soybean saponins stimulate AMP-activated protein kinase but decrease the activity of the target of rapamycin (TOR). Soybean saponins increased mRNA expression of growth marker genes, including growth hormone, insulin-like growth hormone factor 1, growth hormone reactor A, and growth hormone receptor B. However, they decreased insulin-like growth factor-binding protein at mRNA and protein levels. Further research on saline tilapia is essential to do.

CONCLUSION

Based on the study results, it can be concluded that feed enrichment using *Melastoma malabathricum* leaf extract positively affects the growth and survival of saline tilapia fry. The *Melastoma malabathricum* leaf extract of as much as 1 g/kg feed gave the best results for the tilapia fry growth parameters in brackish water (8 ppt).

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