

## **Effectivity of karamunting *Melastoma malabathricum* leaves in inhibiting ovarian development of Nile tilapia *Oreochromis niloticus***

### **Efektivitas daun karamunting *Melastoma malabathricum* dalam menghambat perkembangan ovarium ikan nila *Oreochromis niloticus***

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#### **ABSTRACT**

Tilapia gonads mature quickly before reaching market size, caused by a diverting of feed energy from growth to reproduction. As a result, somatic growth is disrupted to achieve market size, the operational costs are high, and the rearing period is longer. This study aims to evaluate the ability of karamunting leaf extracts to inhibit the development of tilapia gonads. This study used a complete randomized design with four treatments and three replications, namely 0 mg/kg, 25 mg/kg, 50 mg/kg, and 100 mg/kg of karamunting leaf extract. Tilapia fish weighed 13–14 g were kept in an aquarium measuring 100×60×50 cm with a stocking density of 20 fish/ aquarium. Fish were fed twice a day at 8 a.m and 5 p.m in at satiation. Sampling was carried out at the beginning of the study, on day 30<sup>th</sup> and day 60<sup>th</sup>. On day 30<sup>th</sup> the result showed that the best dose in inhibiting the development of fish gonad was 100 mg/kg of karamunting leaf extract that was  $1.15 \pm 0.19\%$  and the daily growth rate was increased at  $2.22 \pm 0.06\%$ . On day 60<sup>th</sup>, the best dose in inhibiting gonad development was 25 mg/kg of karamunting leaf extract, which was  $2.49 \pm 1.24\%$  and the daily growth rate was increased amount  $3.26 \pm 0.06\%$ .

**Keywords:** Extract, karamunting leaves, gonad development, tilapia

#### **ABSTRAK**

Gonad ikan nila cepat berkembang sebelum mencapai ukuran pasar, menyebabkan pengalihan energi pakan dari pertumbuhan ke reproduksi. Akibatnya, pertumbuhan somatik terganggu sehingga untuk mencapai ukuran pasar biaya operasional tinggi dan masa pemeliharaan lebih lama. Penelitian ini bertujuan mengevaluasi ekstrak daun karamunting dalam menghambat perkembangan gonad ikan nila. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan; yaitu 0 mg/kg, 25 mg/kg, 50 mg/kg, dan 100 mg/kg ekstrak daun karamunting. Ikan nila berukuran 13–14 g dipelihara di akuarium berukuran 100×60×50 cm dengan padat tebar 20 ikan/akuarium. Ikan diberi makan dua kali sehari pada jam 8 pagi dan 5 sore dengan at satiation. Pengambilan sampel dilakukan pada awal penelitian, hari ke-30 dan hari ke-60. Hasil pada hari ke-30 menunjukkan bahwa dosis terbaik dalam menghambat perkembangan gonad pada ikan uji adalah 100 mg/kg ekstrak daun karamunting yaitu  $1.15 \pm 0.19\%$  dan meningkatkan pertumbuhan harian  $2.22 \pm 0.06\%$ . Pada hari ke-60 dosis terbaik dalam menghambat perkembangan gonad adalah 25 mg/kg ekstrak daun karamunting yaitu  $2.49 \pm 1.24\%$  dan meningkatkan pertumbuhan harian  $3.26 \pm 0.06\%$ .

**Kata kunci:** Daun karamunting, ekstrak, ikan nila, pertumbuhan gonad

## INTRODUCTION

*Tilapia Oreochromis niloticus* is one of the most prospective freshwater commodities. It is introduced in Africa in 1969 and now it is the most popular freshwater aquaculture species in Indonesia. It is majorly consumed because of the taste and high protein content. The high cost of commercial feed still becomes a problem. Commercial feed takes up to 50% of the total production cost (Sari *et al.*, 2017; Amin *et al.*, 2020). The common problem of tilapia culture is its high reproduction. It frequently disrupts the grow-out phase of tilapia because of the energy allocation supposedly utilized for growth instead of reproduction (Kareem *et al.*, 2016; Kapinga *et al.*, 2018). Female tilapia has the parental care instinct to brood the eggs inside her mouth for 10–12 days (Tamamdusturi & Basuki, 2012). During the brood, a broodstock will not be eating so the growth will be slow and tend to decrease (Beaven & Muphosi, 2012).

Srisakultiew and Komonrat (2013) also Dagne *et al.* (2013) stated that male tilapia grows faster than females. In female tilapia, the energy allocation for reproduction is higher than in males (Barades *et al.*, 2020). As a result, the harvested size varied and the objected weight is not achieved despite the high cost of production. Data showed that the male monosexual population of tilapia brought a greater production compared on mix sexual population (Mulyani *et al.*, 2012; Deswira *et al.*, 2015; Djuanaedi *et al.*, 2016). Commonly, the production of male mono sexual tilapia is managed using sex reversal technology by utilizing a hormone treatment, such as soaking in  $17\alpha$ -methyltestosterone. Unfortunately, currently,  $17\alpha$ -methyltestosterone utilization is limited by the government through Peraturan Menteri Kelautan dan Perikanan no 39/PERMEN-KP/2015 about controlling medical, chemical, and contaminants in fish, especially in the consumed aquaculture species (KKP, 2015).

The plant-based matter is majorly used in aquaculture. The plant-based compound has a similar physiological activity to the animal-based compound. One of the abilities of plant-based compounds is to both increase and inhibit the reproduction performance of fish. Malabar melastome *Melastoma malabathricum* is one of the plants that inhibit fish reproduction. Farizah (2017) presented that malabar melastome injection in 2 mg/kg of dosage can slower the reproduction of *Scylla* spp. This plant is known as wild weeds and is commonly found in Asia and the Pacific Islands. It is also considered an herb by the locals (Joffry *et al.*, 2012). Laboratorium test concluded that this plant contains flavonoids, tannin, saponin, phenolic, steroid, and triterpenoid (Farizah, 2017).

Winarno and Sundari (1997) stated that flavonoids can constrain aromatase hormone which is an enzyme that catalyzes androgen conversion to estrogen to increase testosterone. Flavonoid utilization through feed in a particular dosage will inhibit the aromatase activity and it will affect estrogen and androgen content (Gabriel *et al.*, 2015; Abaho *et al.*, 2021).

## MATERIALS AND METHODS

This study was conducted for 60 days, starting in May to July 2018. Proximate analysis was managed in the Fish Nutrition Laboratory. Fish rearing was handled in the rearing installation of Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Meanwhile, the phytochemical test was done in Balai Penelitian Tanaman Rempah dan Obat (BALITRO) Bogor.

### Experimental design

This study applied a completed randomized design which consisted of four treatments. Every treatment was replied to three times. Details about treatment were presented below in Table 1.

Table 1. The oral treatment of Malabar melastome.

Treatment	Description
0 mg/kg	Feed without Malabar melastome treatment
25 mg/kg	A feed with 25 mg/kg of Malabar melastome treatment
50 mg/kg	A feed with 50 mg/kg of Malabar melastome treatment
100 mg/kg	Feed with 100 mg/kg of Malabar melastome treatment

### Malabar melastome leaves extraction

Fresh leaves and physiologically matured were selected from the Bogor region. After being picked, leaves were washed thoroughly and weighed. Moreover, leaves were air-dried for five days in an open area but spared from direct sunlight. The completed dry leaves were processed in powder form using a blender and stored in tightly closed storage. Extraction is the process to withdraw a dissolved compound from a material using a certain solvent (Mukhriani, 2014). The extraction process is dependent on the isolated compound and the solvent. Malabar melastome extraction applied the soaking method using ethanol 80%. A 100 g of melastome leaves powder was put in a dark Erlenmeyer and soaked with 600 mL of ethanol 80%. The tube was closed tightly and stored at room temperature and spared from sunlight for 72 hours while stirred every eight hours. The liquid resulting from the ethanol soaking was filtered using filter paper. Furthermore, the dregs were repeatedly soaked using the same method twice with the same solvent as many 200 mL. The liquid obtained from the soaking procedure was gathered and then steamed at 40°C until the liquid became more viscous.

### Feed coating

The viscous extract was used to coat the experimental feed according to the dosage. Feed coating was conducted using eggs, water, the melastome extract, and progol. Those ingredients were mixed using a blender thoroughly. The mixture was evenly sprayed to the commercial feed using the manual sprayer. Moreover, the feed was dried in an oven (40°C).

### Rearing container preparation

As many 12 units of aquariums sized 100×60×50 cm were used as rearing containers. The aquariums were cleaned and dried two days beforehand. Each of the aquariums was equipped with an aeration system to provide oxygen supply during the study.

### Experimental fish

The experimental fish was Nile tilapia *Oreochromis niloticus* was selected from the Instalasi Penelitian Plasma Nutfah Perikanan Air Tawar, Cijeruk, Bogor, West Java. The experimental tilapia was sized in 13–14 g and they were reared in 20 fish in each container.

### Fish feeding and rearing

Feeding was done for 60 days using the coated feed. The fish was fed using at satiation feeding method twice a day at 8.00 and 17.00. During the study, all the rearing containers were siphoned every day and the water discharge was done 70% every five days.

### Experimental parameters

The tested parameters in this study consisted of gonad maturation index (GMI), hepatosomatic index (HSI), absolute weight (AW), growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), gonadal histology, and eggs diameter.

### Data analysis

Data were tabulated using Microsoft Excel 2016. GMI, HIS, AW, GR, FCR, and SR were analyzed with analysis of variance in 95% confidence level using SPSS 22. A posthoc test was also done when a significant difference existed. The egg diameter was measured using software named IMAGE-J v1.8. Gonad histology, GMI, and gonad development were presented descriptively.

## RESULTS AND DISCUSSION

### Results

At the first 30 days, then 100 mg/kg treatment showed the highest absolute weight and daily growth rate and it was significant among the 25 and 50 mg/kg treatment, but it was not compared to the control treatment. The lowest GMI was spotted on the 25 mg/kg and 100 mg/kg. It differed significantly compared to the control and 50 mg/kg treatment. Meanwhile, the survival rate, FCR, and HSI did not differ significantly amongst treatments ( $P>0.05$ ) (Table 2).

At the end of the study, the 25 mg/kg presented the highest growth rate and it was different significantly compared to the 50 and 100 mg/kg treatment, yet it was not significant with the control ( $P>0.05$ ). The lowest survival rate was noted in the 50 mg/kg treatment ( $P<0.05$ ), while the lowest FCR, GMI, and HSI were found in the 25 mg/kg treatment compared to others (Table 3).

The egg diameter was observed on days 30 and 60. According to the observation result on day 30, the egg diameter in the control treatment was dominated by eggs diameter ranging from 1.41–1.66 mm, while the eggs diameters ranging from 0.11–1.92 mm were majorly found in the 25 mg/

kg treatment. In the higher dosage treatments (50 mg/kg), the diameter of the eggs majorly ranged from 0.11–0.62 mm and 1.67–2.18 mm. The lowest egg diameter classification was observed from the 100 mg/kg treatment that ranged from 0.11–1.14 mm (Figure 1).

Based on the egg measurement on day 60, the

control treatment was dominated by size class 1.57–2.14, whereas the 25 mg/kg treatment had a fairly distributed size class from 0.12–2.14. However, in the higher treatment (50 mg/kg), the egg diameter size class was relatively wider (1.28–1.56) compared to the control treatment and 25 mg/kg. The lowest size class was shown

Table 2. Initial biomass, final biomass, absolute weight, daily growth rate, feed conversion ratio, survival rate, gonad maturation index, and hepatosomatic index of tilapia that were fed using melastome extract for 30 days.

Tested parameters	Treatments			
	0 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
W0 (g)	14.13 ± 0.28 <sup>a</sup>	14.09 ± 0.14 <sup>a</sup>	13.90 ± 0.14 <sup>a</sup>	13.98 ± 0.13 <sup>a</sup>
Wt (g)	50.85 ± 1.80 <sup>a</sup>	48.74 ± 1.69 <sup>b</sup>	45.21 ± 1.90 <sup>b</sup>	52.37 ± 2.21 <sup>a</sup>
BM (g)	36.72 ± 1.72 <sup>ab</sup>	34.64 ± 1.82 <sup>bc</sup>	31.31 ± 1.81 <sup>c</sup>	38.39 ± 2.11 <sup>a</sup>
SGR (%/day)	2.16 ± 0.06 <sup>ab</sup>	2.09 ± 0.07 <sup>bc</sup>	1.98 ± 0.06 <sup>c</sup>	2.22 ± 0.06 <sup>a</sup>
FCR	1.05 ± 0.08 <sup>a</sup>	1.02 ± 0.09 <sup>a</sup>	1.08 ± 0.04 <sup>a</sup>	1.02 ± 0.02 <sup>a</sup>
SR (%)	100.00 ± 00 <sup>a</sup>	100.00 ± 00 <sup>a</sup>	100.00 ± 00 <sup>a</sup>	100.00 ± 00 <sup>a</sup>
GMI (%)	2.70 ± 0.06 <sup>b</sup>	1.36 ± 0.26 <sup>a</sup>	2.76 ± 0.44 <sup>b</sup>	1.15 ± 0.19 <sup>a</sup>
HSI (%)	2.63 ± 0.27 <sup>a</sup>	2.49 ± 0.07 <sup>a</sup>	2.42 ± 0.14 <sup>a</sup>	2.42 ± 0.19 <sup>a</sup>

Note: Different superscript behind the deviation standard indicates significant difference (P<0.05). W0=initial weight, Wt=final weight, BM=absolute final weight, SGR=specific growth rate, FCR=feed conversion ratio, SR=survival rate, GMI=gonad maturation index, HSI= hepatosomatic index.

Table 3. Initial biomass, final biomass, absolute weight, daily growth rate, feed conversion ratio, survival rate, gonad maturation index, and hepatosomatic index of tilapia that were fed using melastome extract for 60 days.

Tested parameters	Treatments			
	0 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
W0 (g)	14.13 ± 0.28 <sup>a</sup>	14.09 ± 0.14 <sup>a</sup>	13.90 ± 0.14 <sup>a</sup>	13.98 ± 0.13 <sup>a</sup>
Wt (g)	95.19 ± 18.18 <sup>b</sup>	96.27 ± 2.72 <sup>a</sup>	83.24 ± 14.69 <sup>b</sup>	86.95 ± 17.05 <sup>b</sup>
BM (g)	81.06 ± 18.15 <sup>a</sup>	82.18 ± 2.87 <sup>a</sup>	69.35 ± 14.61 <sup>c</sup>	72.96 ± 16.99 <sup>b</sup>
SGR (%/day)	3.21 ± 0.34 <sup>a</sup>	3.26 ± 0.06 <sup>a</sup>	3.01 ± 0.31 <sup>b</sup>	3.07 ± 0.34 <sup>b</sup>
FCR	1.09 ± 0.70 <sup>a</sup>	1.08 ± 0.09 <sup>b</sup>	1.15 ± 0.13 <sup>a</sup>	1.20 ± 0.14 <sup>a</sup>
SR (%)	100.00 ± 00 <sup>a</sup>	96.67 ± 2.89 <sup>a</sup>	95.00 ± 8.66 <sup>b</sup>	100.00 ± 00 <sup>a</sup>
GMI (%)	3.02 ± 0.56 <sup>b</sup>	2.49 ± 1.24 <sup>c</sup>	3.17 ± 0.18 <sup>a</sup>	3.69 ± 0.39 <sup>a</sup>
HSI (%)	2.22 ± 0.57 <sup>a</sup>	2.04 ± 0.46 <sup>b</sup>	2.75 ± 0.38 <sup>a</sup>	2.25 ± 0.24 <sup>a</sup>

Note: Different superscript behind the deviation standard indicates significant difference (P<0.05). W0=initial weight, Wt=final weight, BM=absolute final weight, SGR=specific growth rate, FCR=feed conversion ratio, SR=survival rate, GMI=gonad maturation index, HSI= hepatosomatic index.

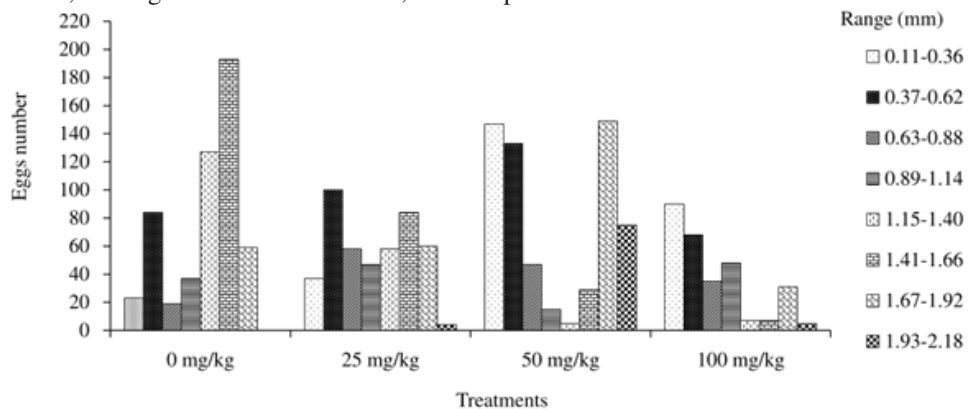


Figure 1. Frequency distribution of egg diameters on day 30.

by the 100 mg/kg treatment (0.12–0.69) (Figure 2).

Histology observation in the initial study found that a 12–15 g of tilapia had the gonad already and the oogonia were started to be seen in the gonad. On day 30, it was observed that the gonad development was started to occur, but the control treatment experienced it faster than the others. On day 60, it was known that all tested tilapia had had their gonad developed already (Table 4).

**Discussion**

The addition of melastome extract in a 25 mg/kg dosage could inhibit the gonad maturation of

tilapia. It was proved by the lowest GMI ( $2.49 \pm 1.24\%$ ) compared to the other treatments. Active compounds were suspected of inhibiting the gonad maturation process, such as flavonoids, triterpenoids, steroids, tannin, and saponin. Winarno and Sundari (1997) stated that flavonoids can lower the aromatase enzyme so that the vitellogenesis will be decreased and cause a low GMI. In addition, a common herb that contained an aphrodisiac compound and acts as an inducer in gonad maturation was reported to have an anti-fertility impact (Gabriel *et al.*, 2015; Abaho *et al.*, 2021). In a female individual, a certain

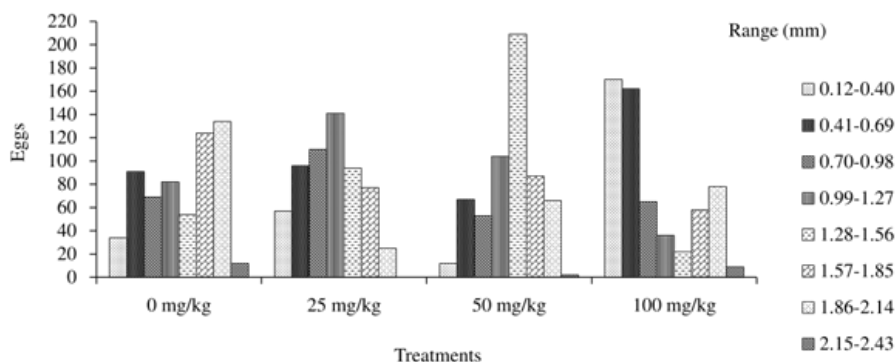


Figure 2. Frequency distribution of egg diameters in day 60.

Table 4. Histology observation on days 0, 30, and 60.

Treatments	Day-0	Day-30	Day-60
0 mg/kg			
25 mg/kg			
50 mg/kg			
100 mg/kg			

Note: Oogonia (Og), oocyte (Oc), nucleus (N), yolk vesicle (YV), yolk globule (YG), thecal layer (T), and zona radiata (ZR).

dosage of herb affected fertility by lowering the estrogen and progesterone level (Adewale *et al.*, 2014).

This study explained that the 25 mg/kg treatment showed a lower HIS ( $2.04 \pm 0.46\%$ ) compared to other treatments. On the other hand, the control treatment showed a higher result for GMI ( $3.02 \pm 0.56\%$ ) and HIS ( $2.22 \pm 0.57\%$ ). It explained that the gonad development would occur normally without melastome extract. Estradiol-17 $\beta$  concentration in the granulosa layer elevated and induced the liver to boost vitellogenin synthesis followed by the increase of HSI (Reading & Sullivan, 2017). It was supported by Farizah (2017) who stated that *Scilla olivacea* injected using melastome extract in a dosage 2 mg/kg had a slow gonad maturation process.

This study particularly treated the fish using melastome extract on day 60 with dosages of 50 mg/kg and 100 mg/kg. Instead of inhibiting the gonad maturation, the gonad matured faster. Both treatments had a higher result compared to the control. It was presumably caused by the active compounds, i.e. phytoestrogens that induce gonad maturation. According to Farizah (2017), melastome extract contained bioactive agent I promoting reproduction performance of *Scilla olivacea*. Melastome extract also has the phytoestrogens that have a similar mechanism to the common estrogen that influences the reproduction process, both stimulating and inhibiting (Putra & Razai, 2017).

The low GMI and HIS in the 25 mg/kg treatment indicated that gonad maturation was inhibited because it lacks energy allocation for reproduction so the energy was utilized in somatic development. It was shown by the highest growth result in the 25 mg/kg treatment ( $3.26 \pm 0.06\%$ /day) compared to the control. Moreover, the addition of melastome extract resulted in an efficient level ( $1.08 \pm 0.09$ ), whereas the 100 mg/kg treatment had the highest FCR ( $1.20 \pm 0.14$ ). It could be seen by the GMI value ( $3.69 \pm 0.39\%$ ) and it could be caused by the nutrient flow that is majorly utilized in gonad maturation. Mukti (2016) stated that somatic development in a reproductively unmaturing tilapia will be faster compared to reproductively matured tilapia.

The addition of melastome in a dosage of 25 mg/kg caused the egg diameter to spread evenly. The control treatment showed an averagely large size of egg diameter. Reading dan Sullivan (2017) described that follicle-stimulating hormone (FSH) will stimulate theca cell to release the testosterone

that induces the granulosa cell to produce estradiol-17 $\beta$  incorporate with the aromatase enzyme. Estradiol-17 $\beta$  that was produced will be released into the bloodstream and it will induce the liver to produce vitellogenin. Vitellogenin will be released again into the bloodstream and selectively absorbed by the oocyte. The result of vitellogenesis will cause the development of egg diameter and gonad. Winarno and Sundari (1997) stated that flavonoids could inhibit aromatase enzymes so that the oocyte growth will be slower. Natural ingredients utilization in aquaculture is considered promising because it is safer for both the commodity and the environment and it is easily degraded (Olusola *et al.*, 2013; Reverter *et al.*, 2014).

## CONCLUSION

Melastome leaves extract (*Melastoma malabathricum* L.) worked as a bioactive agent in the tilapia reproduction process. Feeding using melastome leaves extract could inhibit the gonad maturation after 30 days of feeding using a dosage of 100 mg/kg and after 60 days using a dosage of 25 mg/kg.

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