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The Side Effect of the *Melastoma malabathricum* L Ethanol Extract on the Gonad Maturation of Female Orange Mud Crab (*Scylla olivacea*)

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ABSTRACT

The medicinal plants in aquaculture are currently being studied quite extensively by researchers. Medicinal plants can act as an inducer or aphrodisiac and an antifertility agent in gonad maturation. The aimed of the experiment was to known side effects of the herb *M. malabathricum* L. on maturation process in female mud crab (*S. olivacea*). This study used 40 crabs with an average weight of 200-250g. The experiment consists of two treatment groups. The controls group (A) that received 100 µl of aquadest and group treatment (B) received 100 µl of 2 mg/g of ethanol extract *M. malabathricum* L. were studied. For 5, 10, 15, and 20 days of experiment, crabs were anesthetized and sacrified. The ovary was collect for GSI and histology. The end of experiment days (20 day), the hemolymph was collect to used ELISA assay. The result showed, significant differences in treatment group and control groups ($p<0.05$) in terms the macroscopic morphology of ovary, the mean GSI and the diameter of oocytes. Histological showed the influence of extract *M. malabathricum* L. on inhibited the ovary development compared with the control groups. Based on this results, it is concluded that ethanol extract of *M. malabathricum* L. leaves at a dose of 2 mg/g act as an antifertility agent for ovary maturation of *S. olivacea*.

1. Introduction

Indonesia has 1300 known species of medicinal plants (Sangat *et al.* 2000). Medicinal plants are well known by the locals and have been traditionally utilized since long ago to treat various diseases and used as tonics. The use of medicinal plants themselves in the field of aquaculture are currently being studied quite extensively by researchers (Citarasu 2010). Medicinal plants have tonic-like properties to boost the immunity system (Sivaram *et al.* 2004; Minomol 2005; Citarasu *et al.* 2006), can act as a promotor in accelerating growth rate (Ashraf and Goda 2008), an appetite stimulator in increasing feed consumption (Jayaprakas and Euphrasia 1996; Kim *et al.* 1998; Venketramalingam *et al.* 2007), an inducer or aphrodisiac in gonad maturation (Babu 1999; Linan-Cabello *et al.* 2004), as an antifertility agent (Jegede 2011; Ghosal and Chakraborty 2014; Gabriel *et al.* 2015), an antimicrobial (Dabur 2004; Khan *et al.* 2004; Chitmanat *et al.* 2005) and antistress agent (Wu *et al.* 2007; Hsieh *et al.* 2008; Ardo *et al.* 2008). However, scientific testing of medicinal plants in aquatic organisms is still rare even though there is a great potential for medicinal plants in Indonesia which naturally contain active ingredients which have promising prospect (bioprospecting) to be developed as a product in increasing aquaculture production. One of the potentials of medicinal plants is as an antifertility agent. Generally, medicinal plants which function as an aphrodisiac and inducer in gonad maturation are also reported to have antifertility properties (Raj *et al.* 2011). Medicinal plants can function as an antifertility and abortive agent (Devi 1995; Obaroh and Chionye-Nzeth 2011; Gabriel *et al.* 2015).

The medicinal plant compounds that have phytoestrogenic properties are isoflavonoids (flavonoids and isoflavons), coumestans (coumestrol),

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lignan (Lehtinen *et al.* 2001), antraquinone (Matsuda *et al.* 2001), chalcone (Rafi *et al.* 2000), flavone (Milligan *et al.* 1999), and saponin (Chan *et al.* 2002). Phytoestrogens, which have a structure similar to the steroid hormone, estradiol (E2), in animals (Lehtinen *et al.* 2001). Phytoestrogens have the same effect as estrogen which influences the reproduction process, both stimulating and inhibiting the reproduction process (Tsai *et al.* 2000; Trant *et al.* 2001; Andersen *et al.* 2003). Phytoestrogens have a low molecular weight so they can easily pass through cell membranes then bind with estrogen receptors (ER) and triggering either an estrogenic or antiestrogenic effect (Adlercreutz 1998; Lehtinen *et al.* 2001; Ososki and Kennelly 2003). The estrogenic or antiestrogenic effect in animals depends on the ratio between endogenic estrogen, aromatase activity, species, reproduction status, exposure duration, and administration method (oral or parenteral) (Monteiro *et al.* 2000; Bennetau-Pelissero *et al.* 2001; Green and Kelly 2009). The administration of sub-chronic and chronic dosages of medicinal plants affects fertility in both male and female individuals (Raj *et al.* 2011). In male individuals, there is a decrease in testosterone levels and concentration and spermatozoa motility and sexual disfunction (Cherdshewasart *et al.* 2008). In female individuals, antifertility disorders are caused by mechanisms such as interruptions of the oestrus cycle, anti-estrogenic agents, anti-implantation agents, and abortive agents (Devi *et al.* 2015). The decrease in estrogen and progesterone levels (Adewale *et al.* 2014) and oestrus cycle disorders in rats cause abortions (Raj *et al.* 2011). The results reported for humans and mammals are also applicable for aquatic organisms (fish). A number of herbal extracts (medicinal plants) have the ability to induce antifertility, abortions, and sex change in aquatic organisms (Gabriel *et al.* 2015).

Melastoma malabathricum L. is a plant from Asia and the Pacific Islands and is known as a medicinal plant by the local people (Joffry *et al.* 2012). This herb is used to overcome reproductive issues by increasing fertility and strengthening the womb (Koay 2008), increasing the concentration and motility of spermatozoa and raising the testosterone level in rats (Balamurugan *et al.* 2013), and as an inducer in the ovary maturation acceleration process in mud crab *S. olivacea* (Farizah *et al.* 2016; Farizah *et al.* 2017). This study was aimed to evaluate the effect of the ethanol extract of *M. malabathricum* L. on the reproduction of female orange mud crabs (*S. olivacea*) as an inhibitor agent in the ovary maturation process.

2. Materials and Methods

2.1. Plant Material

The materials were used are fresh leaves of *Melastoma malabathricum* L. and they were collected from North Samarinda, East Borneo, Indonesia. The plants used in this study were identified by Indonesian Institute of Sciences (LIPI) Bogor.

2.2. Preparation of Ethanol Extracts *Melastoma malabatricum* L.

The leaf extraction was conducted based on Balamurugan *et al.* (2013) with modification procedure. The extraction used maceration technique with 80% ethanol solvent (1:5). The maceration was conducted with 100 g leaf powder on chamber glass and 500 ml 80% ethanol as a solvent. The maceration procedure was repeated for three times (3 x 24 hours) at room temperature with dark environment, then extract was filtered. The ethanol extract was concentrated in a rotary evaporator at 40ºC of the temperature. The ethanol extract was used for terpenoid or steroid compound testing based on Goad and Toshihiro method (1997).

2.3. Experiment Animal

The immature female mud crabs (*S. olivacea*) were used for in vivo assay. The body weight of female mud crabs were between 200-250 g with carapace width between 10-13 mm. Mud crab adaptations was performed in cage for three days. Each cage consisted of one female mud crab on each group. Each group was performed with five replicates.

2.4. Experimental Design

The female mud crabs were divided into 2 groups consisting of 5 animals.

Group A :Mud crab received 100 µl aquadest soluble for 5,10,15, and 20 days (control), by injection.
Group B :Mud crab received 100 µl ethanol extract of leaf of *Melastoma malabathricum* at the dose of 2 mg/g body weight for 5,10,15, and 20 days, by injection.

The injections were performed using 1 ml syringe with 27 g needle at the internode of swimming leg of crab. Each crab was injected, with concentration of 1/10 mg/g of body weight (Fujaya *et al.* 2011). After that, the mud crabs were placed back into the

bamboo cage after receiving the injection. Female mud crabs were monitored for 20 days in the bamboo cage. During the culture period, the mud crabs were fed with rough fish and squid approximately 10% of crab body weight for two times per day. Water quality measurements were consisted of: dissolved oxygen (DO), pH, salinity and temperature.

In vivo assays were observed by mud crab ovarian maturation stages that were described based on ovarian morphology, gonad somatic index (GSI), hormonal assay, oocyte diameter size, and ovary histology evaluation.

2.4.1. Ovarian Maturation Stages

The observation of ovarian maturation stages was conducted morphologically (macroscopic). Data was collected every 5 days of treatment for ovarian maturation stages observation. This step followed the procedure of Islam *et al.* (2010). After the observation, ovarian tissue was dissected in formaldehyde 4% for histology preparation.

2.4.2. Gonad Somatic Index (GSI) Measurement

Gonad Somatic Index (GSI) and Hepatosomatic Index (HSI) were calculated based on ovary and hepatopancreas weight. The ovaries and hepatopancreas were dissected out from the crab. GSI and HSI were calculated as followings:

GSI = gonad weight / body weight x 100

HSI = hepatopancreas weight / body weight x 100

2.4.3. Hormone Levels Measurement

Estradiol 17β hormone levels were analyzed from hemolymph sample with ELISA method (Estradiol 17β-ELISA test kit) according to the manufactured instruction. Hemolymph samples were taken from coxa (the forth walking leg of crab) through internode. About 1 ml hemolymph samples were taken using 1 ml syringe which have rinsed with anticoagulant. Then, serum will be separated by centrifugation at 2500 rpm for 15 min and stored at -18ºC to -20 ºC (Pattiasina *et al.* 2010). Standard range for making standard curve is from 1-25 µg/dl. For concentration accuracy of estradiol hormone, each sample was analyzed with duplicate.

2.4.4. Oocyte Diameter Size Measurement

The mean oocyte diameter of mud crabs were conducted on 100 oocyte cell/replicate for each group. The oocyte with nuclei that showed in histological assessments will be used for measurement. The normality of oocyte data was tested with Kolmogorov-Smirnov normality testing using SPSS ver. 16.

2.4.5. Histology

Microscopic observation was performed from histology slide from each treatment. Sample preparations followed standard laboratory protocols for Haematoxylin-Eosin (HE) staining (Kiernan 1981).

2.5. Statistical Analysis

Data was expressed as graph and table using Ms. Excel 2010 and SigmaPlot ver 10. The data was examined statistically by T tests for GSI, estradiol hormone and oocyte diameter at $p<0.05$ (95%). Macroscopic observation and histological analysis were determined descriptively.

3. Results

3.1. The Development of the Morphology of the Orange Mud Crab Ovaries

The macroscopic changes in the morphology of the orange mud crab ovaries can be seen in Figure 1. The results revealed that there were changes in the volume and color of the ovaries collected every five days from each treatment during the 20 days of the experiment. Changes in the size and color of the ovaries demonstrated the phases in the ovary maturation process referred to Islam *et al.* (2010). Results demonstrated an inhibition activity in the ovary maturation process in treatment group B (2 mg/g) (Table 1 and Figure 1). Based on the scoring results on ovary maturity, the ovary phases and ovary morphological characteristics, it showed that the ovaries were undeveloped from day 10, 15, and 20 compared with group A (the control). This signified that the ethanol extract of *Melastoma malabathricum* L. has antifertility agent properties, acting as an inhibitor in the ovary maturation process of orange mud crabs.

Sampling on the fifth day revealed that the ovary status entered the early maturation phase (early maturing) for group A (control) and group B (2 mg/g). This phase is where the primary vitellogenesis begins, and the macroscopic appearance of the ovary which was creamy white changed to light yellow with a tissue thickness was 3-7 mm and occupied 10-20% of the body cavity (Figure 1).

Sampling on the 10th day revealed that group B exhibited signs of inhibition in ovary development (Figure 2a). The ovary's macroscopic appearance was that part of the tissue underwent an incomplete ovary maturation. There was paler or cream colored tissue surrounding the light yellow ovary. This condition differed from Group A (control) which exhibited an ovarian condition reflecting the initial maturation phase (Figure 1, day 10).

Sampling on the 15th day revealed that the morphology of the ovary had just entered the immature phase. In Figure 2b, the ovary is yellowish white. This condition clearly signified that there was an inhibition action on the ovary maturation process in orange mud crabs that received the extract treatment at a dosage of 2 mg/g. A contrasting result was discovered in group A (control) which exhibited signs of a regular ovary development which was in line with the maintenance duration (Figure 1, day 15). On the 15th day, the control showed signs that the ovary entered the primary vitellogenesis phase. Macroscopically, the ovary tissue was yellow to orange and occupied ± 20-75% of the body cavity. This was in line with the statement by Islam *et al.* 2010, that the color of the ovaries change from yellow to orange with a thickness of 7-12 mm and they occupy 20-75% of the body cavity (Figure 1, day 15).

Ovary sampling on day 20 also demonstrated that treatment group B was experiencing a significant retardation in the ovary maturation process compared to group A (control). Macroscopic observations of the crab ovaries revealed that they were in the immature phase (Figure 2c), the structure of the ovaries resembled a thin and transparent line. This finding strongly contrasted the control (A) that exhibited signs that the ovary development had entered the final stage of maturation. The macroscopic appearance of the ovary is that there is a change in the ovaries' color from yellow to orange and they take 20-75% of the body cavity (Figure 1, day 20).

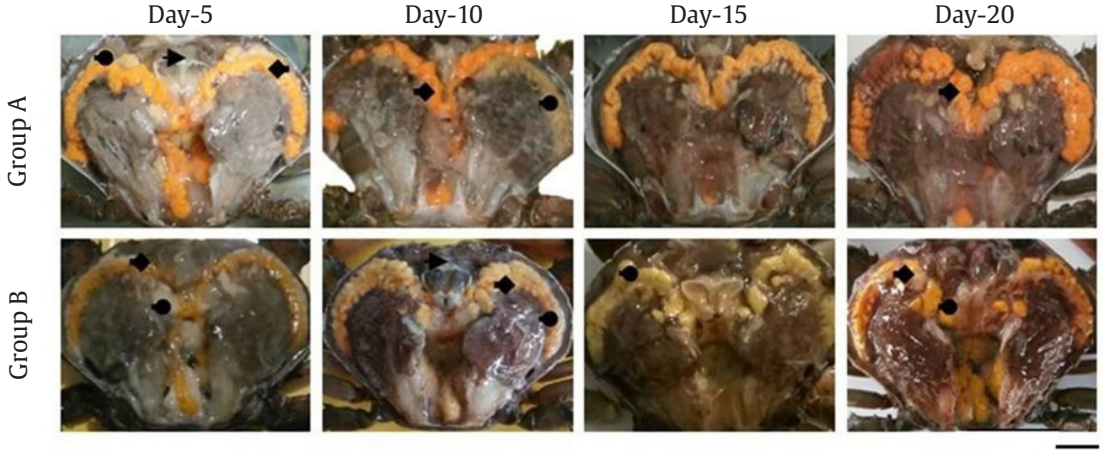

Figure 1. Changes in the ovary morphology of *S. olivacea* in group A (control) and group B (2 mg/g). Notes: 5, 10, 15, 20 (the gonad sample collection days), Notes: = Ovary, = Hepatopankreas, and = Cardiac stomach, bar = (1 cm)

Table 1. The scoring for *S. olivacea* gonad maturation development when given the ethanol extract of *M. malabathricum* L. during the 20 days of research

Treatment	The orange mud crab ovary development											
	Ovary maturation level (OML)				Ovary phase (OP)				Ovary morphological characteristics (OMC)			
	5	10	15	20	5	10	15	20	5	10	15	20
Group A (control)	iii	iii	iii	iv	3	3	3	3	III	III	III	IV
Group B (2 mg/g)	iii	ii	i	i	3	2	1	1	III	II	I	I

OML scoring : (i = *immature*, ii = *developing*, iii = *early maturing*, iv = *late maturing*, and v = *maturation*)
OP scoring : (1 = proliferation , 2 = *pre-vitellogenesis*, 3 = *primary vitellogenesis*, 4 = *secondary vitellogenesis*, and 5 = *tertiary vitellogenesis*)
OMC scoring :

I. The structure of the ovaries resemble a thin and transparent line.
II. The ovaries are white and change to creamy white with a tissue thickness of 2 and 3 mm occupy 1-2 % of the body cavity.
III. The ovaries are creamy white and change to light yellow with a tissue thickness of 3-7 mm and occupying 10-20% of the body cavity.
IV. Change in the ovary color from yellow to orange with a thickness of 7-12 mm and occupying 20-75% of the body cavity.
V. Eggs start to become visible, the ovary changes from orange yellow to orange red with a tissue thickness of 10-20 mm and occupying > 75% of the body cavity.

Number legend: 5, 10, 15, and 20 (sampling day).

3.2. The Gonadosomatic Index (GSI)

Based on the t-test, the administration of the ethanol extract of *M. malabathricum* L. (2 mg/g) had a significant effect on the gonadosomatic index (GSI) ($p > 0.05$). This could be seen in Figure 3. In treatment group B, the GSI range was lower than that of group A (control). This demonstrates that *M. malabathricum* L. extract at a dose of 2 mg/g has potential as an inhibitor that acts in inhibiting the maturation gonad process in orange mud crab compared to the control.

The gonadosomatic index (GSI) range on the maintenance period days 5, 10, 15, and 20 for groups A and B are presented in Figure 4. In group B, the highest average GSI during the duration of the study was 5.93 ± 3.89%, whereas in group A (control), the highest average GSI was 10.17 ± 1.70%. This was twice as high as B. Group B demonstrated a significant slowing ($p > 0.05$) in the crab's ovary maturation process.

3.3. The Testing of the Hormone Estradiol 17ß

The standard solution used in the measurement of the hormone estradiol 17ß is presented in Table 2. The expected concentration and measured concentration of the standard estradiol were the same. The recovery ranged between 97.6 and 104.8%. The concentrations of estradiol in the haemolymph for treatment groups A and B are presented in Figure 5. The results for ELISA revealed that the estradiol concentration in the haemolymph for group A (control) was higher than for group B (2 mg/g). The average concentration of estradiol 17ß in group A was 30.33 pq/ml, whereas for group B it was 19.80 pq/ml.

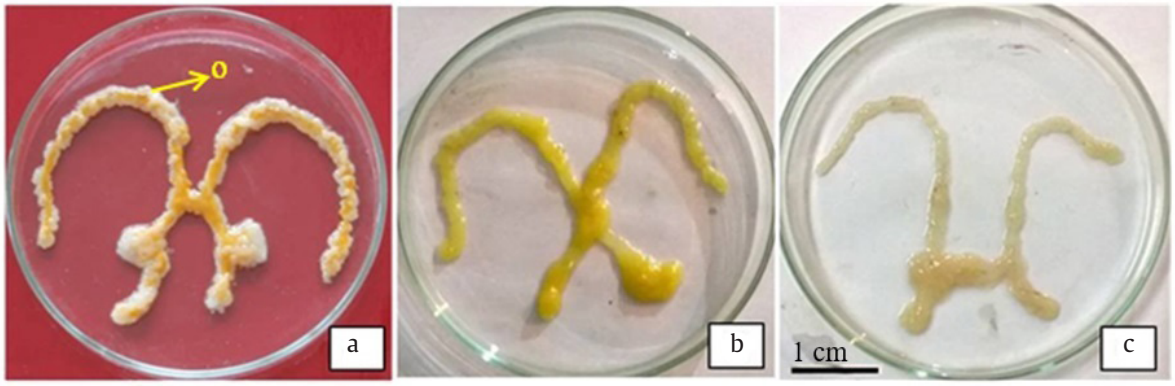

Figure 2. Changes in the ovary morphology of *S. olivacea* for the treatment group B (2 mg/g), Note; a (sampling day 10), b (sampling day 15), and c (sampling day 20), O = Ovary

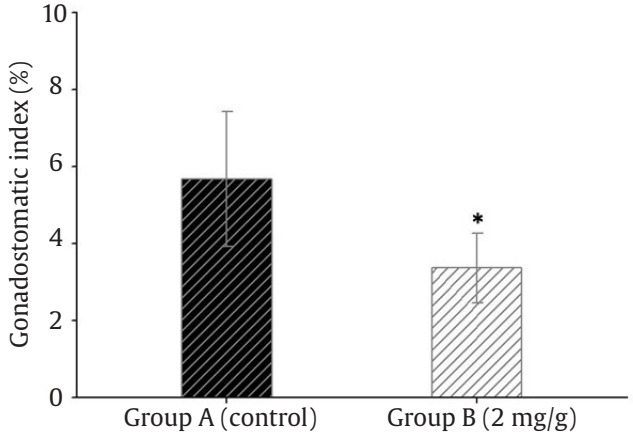

Figure 3. GSI average graph for the orange mud crab (*S. olivacea*), Group A (control) and Group B (2 mg/g). Note: * ($p > 0.05$), n = 40

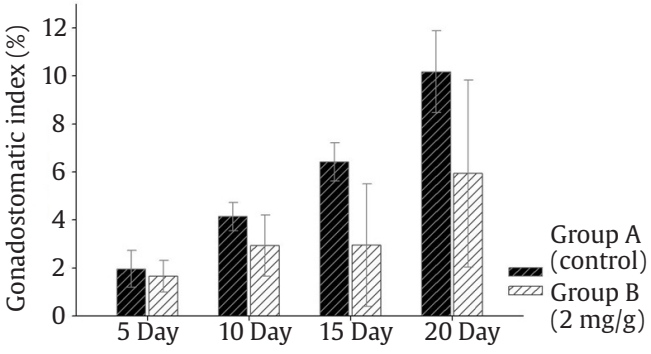

Figure 4. GSI average graph for the orange mud crab(*S. olivacea*), Group A (control) and Group B (2 mg/g), on days 5, 10, 15, and 20 of maintenance (n = 40)

3.4. The Orange Mud Crab's Oocyte Diameter

Based on the t-test, the *M. malabathricum* L. extract had a significant effect on the average cell diameter in orange mud crabs ($p > 0.05$) which can be seen in Figure 6. There was a difference between the treatment with the 2 mg/g ethanol extract of *M. malabathricum* L. (group B) and the treatment that did not include the extract (group A). The oocyte diameter measurement results for groups A and B can be seen in Figure 7. Group A (control) underwent an increase in oocyte size as the maintenance time progressed, resulting in a largest average oocyte diameter of 130.03 ± 7.76%. The growth in oocyte diameter was in line with the increase in GSI for group A, while group B (2 mg/g) demonstrated a largest average oocyte diameter of 43.16 ± 9.03%. The average oocyte diameter for group B was smaller than the average for treatment A; this also had a correlation with the GSI in group B that had a much lower ranger than treatment A (control). The low GSI in group B also determined the ovary maturation level which had not or did not develop.

3.5. The Histological Profile of the Orange Mud Crab Ovary Tissue

The histological profile of the ovaries in group A (control) revealed an oocyte development process that was in accordance to the ovary maturation phase following the maintenance duration (Figure 8a, b, and c). Ovary development with a normal structure was observed on days 10, 15, and 20. The development was in accordance to the gonad maturity level based on the macroscopic morphology and GSI observed (Figure 1 and 3).

The histological profile of the ovaries in treatment group B (2 mg/g) revealed a retardation in the development process in oocytes collected on days 10, 15, and 20 (Figure 9a, b, and c). The histological profile on days 10 and 15 revealed that the ovaries had entered the initial growth phase in primary oocytes. The initial growth began with meiotic cleaving, making the cells primary oocytes. The appearance of the primary oocytes resemble oogonia, but in primary oocytes the ooplasma is more basophilic (Figure 9a and b). Primary oocytes are also larger in size than oogonia, ranging between 15 and 50 µm, and do not undergo mitosis (Brown 2009). On day 20, the histological profile revealed an ovary in the undeveloped phase (Figure 9c). This condition was similar to the phase of the ovary maturity level, namely immature, signified by the large number of oogonia. Macroscopically, ovaries in phase I (immature) are clear and transparent, sometimes difficult to identify and attached to the hepatopancreas (Figure 1, Group B, Day-20). Ovaries in phase I consist of oogonia which are mostly at the periphery of the ovary (Figure 9c). Oogonia are round, 5-10 µm, and the cytoplasm are nearly invisible (Islam *et al.* 2010).

Table 2. Expected concentrations and measured concentrations of standard of DRG Estradiol and their recoveries

Standar	Expected conc (pg/ml)	Measured absorbance	Measured conc (pg/ml)	Recovery (%)
1	25	2.095	24.6	98.3
2	50	1.993	49.8	99.7
3	100	1.775	104.8	104.8
4	250	1.4	254.7	101.9
5	500	1.052	497.5	99.5
6	1000	0.706	975.6	97.6
7	2000	0.402	2044.0	102.2

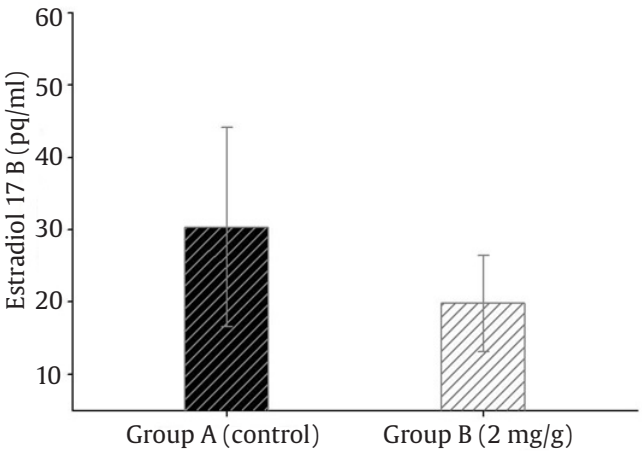

Figure 5. Graph of the estradiol 17 ß level on hemolimp of *S. olivacea* for group A (control) and group B (2 mg/g). *: ($p > 0.05$), n = 6

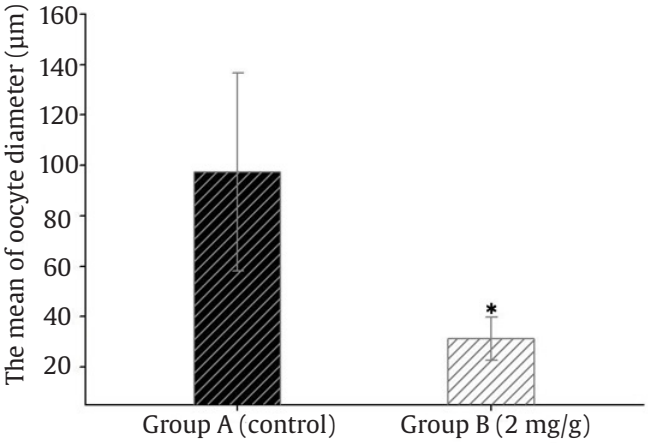

Figure 6. Average oocyte diameter graph for *S. olivacea* given the treatment with *M. malabraticum* L. extract for 20 days of maintenance (n = 40). *: ($P<0.05$)

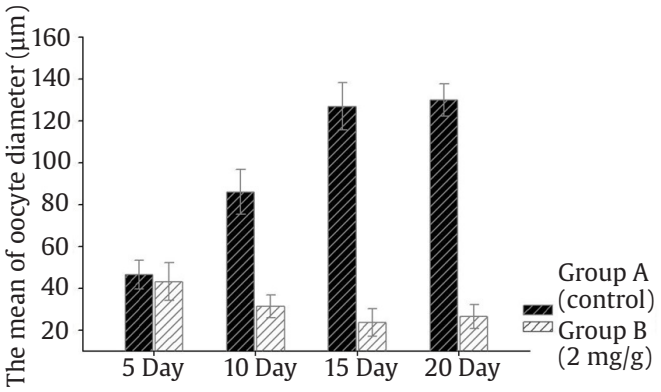

Figure 7. Average oocyte diameter graph for *S. olivacea*, Group A (control) and Group B (2 mg/g), on days 5, 10, 15, and 20 of maintenance (n = 40)

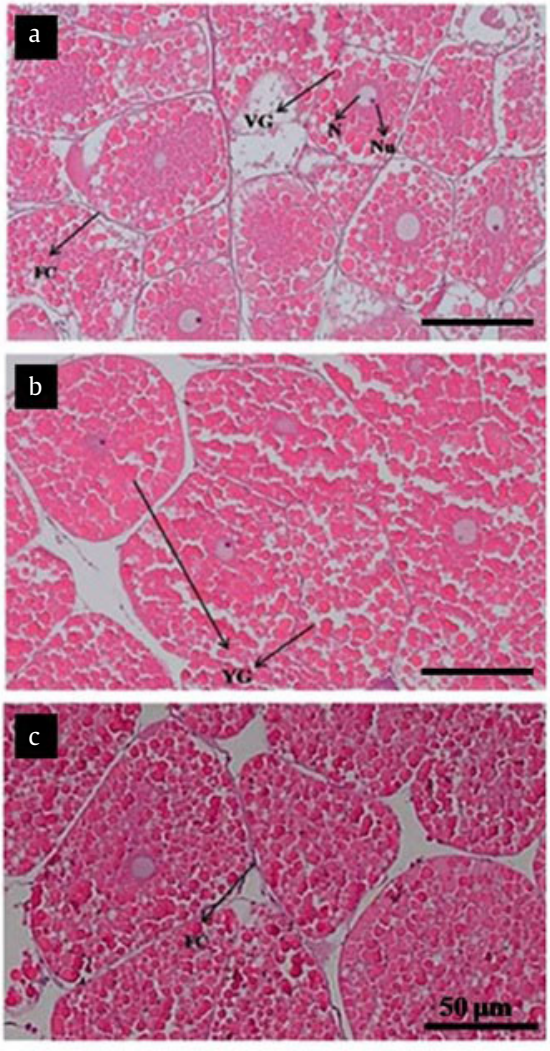

Figure 8. The histological profile of orange mud crab (*S. olivacea*) ovary tissue. caption: treatment group A (control), (a = 10 day, b= 15 day, and c = 20 day). all samples were stained using hematoxylin-eosin. FC = follicle cell, N = nucleus, Nu = nucleolus, and YG = Yolk globule

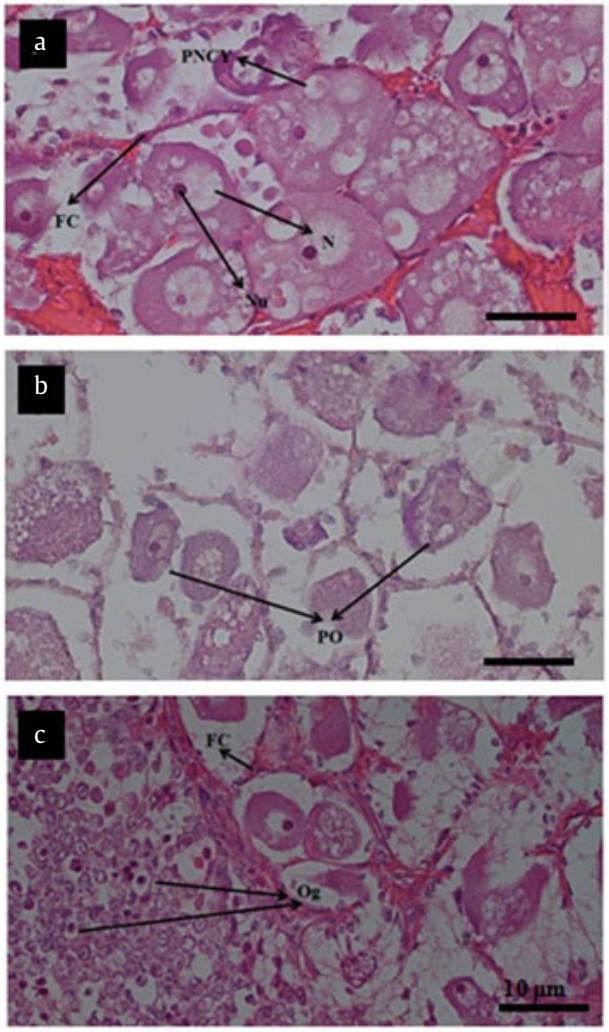

Figure 9. The histological profile of orange mud crab (*S. olivacea*) ovary tissue. caption: treatment group B (2 mg/g), (a = 10 Day, b = 15 Day, and c = 20 Day). all samples were stained using hematoxylin-eosin. FC = follicle cell, N = nucleus, Nu = nucleolus, PO = oocyte primer, PNYC = perinuclear yolk complex, Og = oogonium

4. Discussion

The administration of ethanol extract of *Melastoma malabathricum* L. at a dose of 2 mg/g (Group B) had a effect on the late development of orange mud crab ovaries compared to the control. Macroscopic observations of the ovaries in group B revealed retarded ovary development and a low GSI range compared to group A. These results were consistently recorded in the ovary sampling from day 10, 15, and 20. The estradiol (E2) concentration in haemolymph for group B (2 mg/g) that was collected on day 20 revealed an E2 range of 19.80 pq/ml, indicating that the ovary's maturity level was in the immature phase

and had entered the proliferation phase. In the species *Scylla serrata*, estradiol (E2) that was detected in the haemolymph at the pre vitellogenesis (developing) phase was within the 23 pq/ml range (Warrier *et al.* 2001).

The low average oocyte diameter in group B correlated with the GSI. The low GSI in group B also determined the maturity level of ovaries that have not or did not develop. The histological profile in group B revealed that there was an inhibition in the ovary maturation process on day 10, 15, and 20. The histology of the ovaries at the end of this study (day 20) presented oocytes in the proliferation phase. The diameter of the oocytes ranged between 20 and 45 µm, and clusters oogonia of were discovered in the ovary lobules. Large and clearly visible nucleated follicle cells were found, evidence that group B was still in the immature phase. The follicle cells were larger and were clearly visible in immature gonads (Islam *et al.* 2010). A similar situation was reported in *Scylla serrata* (Quinitio *et al.* 2007). The administration of sub-chronic and chronic doses of medicinal plants influences fertility in female individuals. The results found were in accordance with the reports by several researchers who studied the use of herbal extracts in aquatic animals as an agent to control reproduction (Gabriel *et al.* 2015). A number of herbal extracts that have been tested, namely *M. oleifera* (Ampofo-yeboa 2013), *C. papaya* (Abdelhak *et al.* 2013), *A. vera* (Jegede 2011), *A. indica* (Jegede and Fagbenro 2008), *H. rosasinesis* (Jegede 2010), demonstrated a direct effect on inhibiting gonad maturation through the gonad morphology that did not develop in Nile tilapia. The administration of a number of medicinal plants such as *B. alba* (Ghosal and Charaborty 2014), *Q. saponaria* (Francis *et al.* 2002; Angeles and Chien 2015), *Trigonella. foenum-graecum* (Statlander *et al.* 2008) could regulate the reproduction process in Nile tilapia.

The herbal extract *M. malabathricum* L. at a dose of 2 mg/g via injection resulted in the disruption of the ovary maturation process in *S. olivacea*. Similar results were reported in other studies, such as the addition of the medicinal plant *A. indica* at a dose of 2 g/kg via feed in *T. zilli* resulted in necrotic ovarian tissue (Jegede and Fagbenro 2008). The administration of *H. rosasinesis* at a dose of 3 g/kg via feed in Nile tilapia resulted in a histological profile of gonads that were retarded (Jegede 2010). *A.vera* added to the diet at a dose of 2 ml/kg (Jegede 2011) and *C. papaya* seeds at a dose of 120 g/kg (Abdelhak *et al.* 2013), significantly inhibited the gonad development in Nile tilapia. The inhibition effect demonstrated by medicinal plants which disrupt the reproduction process could be either *irreversible* or *reversible*. It had been reported that large doses of *C. papaya* seeds had a permanent effect (sterility), whereas moderate and low doses had a reversible effect (Abdelhak *et al.* 2013). Based on our investigation, it is concluded that ethanol extract *M. malabathricum* L. at a dose of 2 mg/g, could be used as an antifertility agent that inhibits ovarian development.

Melastoma malabathricum L. is a medicinal plant which has potential in controling the reproductive process in *Scylla olivacea*. There needs to be follow up studies to evaluate the ability of *M. malabathricum* L. as an inhibitor in ovary development, to discover whether the inhibition is reversible or irreversible. Findings from this study is hoped to be applied on other aquatic species and the use of the medicinal plant *M. malabathricum* L. could be an alternative for replaced synthetic hormones. The use of medicinal plants in the field of aquaculture is a very promising opportunity, supported by the advantage of herbal products, being safer for the environment, the aquatic animal and humans because they decompose more readily.

Conflict of interest

There is no conflict of interest.

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References

Abdelhak ME *et al.* 2013. Effects of pawpaw, *Carica papaya* seeds meal on the productive performance and histological characters of gonads in Nile tilapia, *Oreochromis niloticus. Indian J Appl Res* 3:34-37.

Adewale OO *et al.* 2014. Oral administration of leaf extracts of *Momordica charantia* affect reproductive hormones of adult female Wistar rats. *Asian Pac J Trop Biomed* 4:521-524. http://dx.doi.org/10.12980/APJTB.4.2014C939

Adlercreutz H. 1998. Epidemiology of phytoestrogens. *Baillieres Clin Endocrinol Metab* 12:605–623. http://dx.doi.org/10.1016/S0950-351X(98)80007-4

Ampofo-Yeboah A. 2013. Effect of phytochemical feed additives on gonadal development in Mozambique Tilapia, *Oreochromis mossambicus* [Thesis]. South Africa: Stellenbosch University.

Andersen L *et al.* 2003. Vitellogenin induction and brain aromatase activity in adult male and female zebra fish exposed to endocrine disrupters. *Fish Physiol Biochem* 28:319–321. http://dx.doi:10.1023/B:FISH.0000030569.11036.e1

Angeles IP, Chien YH. 2015. Dietary effect of *Quillaja saponaria* and *Yucca schidigera* extract on growth and survival of common carp *Cyprinus carpio* and their antioxidant capacity and metabolic response upon low dissolved oxygen. ISR J AQUACULT BAMID IJA 67.2015.1167. http://dx.hdl.handle.net/10524/49187

Ardo L *et al.* 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture* 275:26–33.

Ashraf MA, Goda S. 2008. Effect of dietary Ginseng herb (Ginsana G115) supplementation on growth, *sinensis* on the immune and physiological responses of white shrimp (*Litopenaeus vannamei*) under *Vibrio alginolyticus* challenge. *Fish Shellfish Immunol* 25:581–588. http://dx doi:10.1016/j.fsi.2008.07.014

Babu MM. 1999. Developing bioencapsulated ayurvedic product for maturation and quality larval production in *Penaeus monodon* [Thesis]. Tirunelveli, India: Manonmaniam Sundaranar University.

Balamurugan K *et al.* 2013. Stimulatory effect of the ethanol extract of *Melastoma malabathricum* L. (Melastomataceae) leaf on the reproductive system of male albino rats. *JAPS* 3:160-165. http://dx.imsear.hellis.org/handle/123456789/151554

Bennetau-Pelissero C *et al.* 2001. Effect of Genistein enriched diets on the endocrine process of gametogenesis and on reproduction efficiency of the rainbow trout, *Onchohynchus mykiss*. *Gen Comp* Endocrinol 121:173–187. http://dx.doi.org/10.1006/gcen.2000.7585

Brown CE. 2009. Ovarian morphology, oogenesis, and changes through the annual reproductive cycle of the female blue crab, Callinectis sapidus Rathbun, in Tampa Bay [Thesis]. Tampa, Florida, United States: University of South Florida.

Chan RY *et al.* 2002. Estrogen-like activity of ginsenoside Rg1 derived from *Panax notoginseng*. *J Clin Endocrinol Metab* 87:3691–3695. http://dx .doi.org/10.1210/jcem.87.8.8717

Cherdshewasart W *et al.* 2008. Androgen disruption and toxicity tests of *Butea superba* Roxb., a traditional herb used for treatment of erectile dysfunction in male rats. *Maturitas* 60:131–137. http://dx.doi:10.1016/j.maturitas.2008.04.011

Chitmanat C *et al.* 2005. Antiparasitic, antibacterial, and antifungal activities derived from a *terminalia catappa* solution against some tilapia (*Oreochromis niloticus*) pathogens. *Acta Hortic* 678:179–182.

Citarasu T *et al.* 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, *Penaeus monodon* with reference to haematological, biochemical and immunological changes. *Fish Shellfish Immunol* 21:372–384. http://dx.doi:10.1016/j.fsi.2006.01.002

Citarasu T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. *Aquacult Int* 18:403–414. http://dx.doi:10.1007/s10499-009-9253-7

Dabur R. 2004. A novel antifungal pyrrole derivative from *Datura metel* leaves. *Pharmazie* 59:568–570.

Devi L. 1995. Effect of Asparagus racemous to improve the survival and reproductive performance in Artemia parthenogenetica [Thesis]. Tirunelveli, India: Manonmaniam Sundaranar University.

Devi P *et al.* 2015. Antifertility activity of medicinal plants on male and female reproduction. *IJPSR* 6:988-1001. http://dx.doi:10.13040/IJPSR.0975-8232.6(3).988-01

Farizah N *et al.* 2016. Accelerated ovarian maturation of mud crab (*Scylla olivacea*) using ethanol extract of *Melastoma malabathricum* L. leaf. *AACL Bioflux* 10:911-921. http://www.bioflux.com.ro/home/volume-10-4-2017

Farizah N *et al.* 2016. Effect of ethanol extract of karamunting (*Melastoma malabraticum* L) leaf on female mud crab (*Scylla* sp.) reproduction system. In: Book of Abstract The synergy of aquaculture stakeholders to strengthen the independency, sustainability, and enviromentally soud fisheries and marine sectors. 1 st International Conference On Aquaculture Biotechnology; 2016 Oct 12; Bogor (ID): IPB. 78 pp.

Francis G *et al.* 2002. Effects of long term feeding *Quillaja* saponins on sex ratio, muscle and serum cholesterol and LH levels in Nile tilapia (*Oreochromis niloticus* L). *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 133:593-603. http://dx.doi.org:10.1016/S1532-0456(02)00167-9

Fujaya Y *et al.* 2011. Molting response, growth, and mortality of mangrove crab (*Scylla olivacea*) supplemented with vitomolt via injection and artificial food. *Ilmu Kelautan* 16:211-218. http://dx.repository.unhas.ac.id/handle/123456789/2887

Gabriel NN *et al.* 2015. Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). *Fish Shellfish Immunol* 44:504-514. http://dx.doi:10.1016/j.fsi.2015.03.002

Gabriel NN *et al.* 2015. Use of herbal extracts for controlling reproduction in tilapia culture: Trends and Prospects - a Review. *ISR J AQUACULT BAMID IJA*_67.2015.1178, 22 pages. http://dx.hdl.handle.net/10524/49194

Ghosal I, Chakraborty BS. 2014. Effects of the aqueous leaf extract of *Basella alba* on sex reversal Nile tilapia, *Oreochromis niloticus* L. *IOSR-JPBS* 9:162-164. http://dx.doi: 10.9790/3008-0922162164

Goad LJ, Toshihiro A. 1997. Analysis of Sterol. London (UK): Blackie Academic and Profesional.

Green CC, Kelly AM. 2009. Effects of the estrogen mimic genistein as a dietary component on sex differentiation and ethoxyresorufin-O-deethylase (EROD) activity in channel *catfish Ictalurus punctatus*. *Fish Physiol Biochem* 35:377–384. http://dx.doi:10.1007/s10695-008-9260-z

Hsieh TJ *et al.* 2008. Effects of Rutin from *Toona* feed utilization, and hematological indices of Nile Tilapia, *Oreochromis niloticus* (L.), fingerlings. *J World Aquac Soc* 39:205–214. http://dx.doi:10.1111/j.1749-7345.2008.00153.x

Islam MS *et al.* 2010. Ovarian development of the mud crab in *Scylla paramamosain* in a tropical mangrove swamps. *JSR* 2:380-389. http://dx.doi.org/10.3329/jsr.v2i2.3543

Jayaprakas V, Euphrasia J. 1996. Growth performance of *Labeo rohita* (Ham.) to Livol (IHF-1000), a herbal product. *Proc Indian Natl Sci Acad B* 63:1–10.

Jegede T, Fagbenro O. 2008. Histology of gonads in *Tilapia zillii* (Gervais) fed Neem (*Azadirachta indica*) leaf meal diets. 8th International symposium on tilapia aquaculture. Cairo, Egypt.

Jegede T. 2010. Control of reproduction in *Oreochromis niloticus* (Linnaeus 1758) using *Hibiscus rosasinensis* (Linn.) leaf meal as reproduction inhibitor. *J Agric Sci* 2:149-154. http://dx .doi: 10.5539/jas.v2n4p149.

Jegede T. 2011. Effects of *Aloe vera* (Liliaceae) on the gonad development in Nile tilapia (*Oreochromis niloticus*) (Linnaeus 1758). In: Fitzsimmons K and Liping L (eds). Proceedings of the 9th International symposiums on tilapia aquaculture. Shanghai, China. Aquafish CRSP. pp. 222-227.

Joffry SM *et al.* 2012. *Melastoma malabathricum* (L.) Smith ethnomedicinal uses, chemical constituents, and pharmacological properties: a review. *Hindawi* 1-48. http://dx.doi:10.1155/2012/258434

Khan S *et al.* 2004. Antifungal activity of *Tamarix dioica* an in vitro study. *Gomal J Med Sc*i 2:2.

Kiernan JA. 1981. Histological and histochemical methods. Theory and practice, Oxford, UK: Pergamon Press. Pp 344.

Kim KJ *et al.* 1998. Effect of glycyrrhizin on rainbow trout *Oncorhynchus mykiss* leukocyte responses. *J Korean Soc Microbiol* 33:263–271.

Koay SS. 2008. Establishment of cell suspension culture of *Melastoma malabathricum* L. for the production of anthocyanin [Thesis]: Pulau Pinang, Malaysia: Universiti Sains Malaysia.

Lehtinen *et al.* 2001. Review of endocrine disrupting natural compounds and endocrine effects of pulp and paper mill and municipal sewage effluents. *The Finnish Environment: Environmental Protection* 447:1-73. http://dx.hdl.handle.net/10138/40427

Linan-Cabello MA *et al.* 2004. Effects of carotenoids and retinol in oocyte maturation of crayfish C*herax quadrucarinatus*. *Aquac Res* 35:905–911. http://dx.doi:10.1111/j.1365-2109.2004.01083.x

Matsuda H *et al.* 2001. Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): structure-requirement of hydroxyanth-raquinones for estrogenic activity. *Bioorg Med Chem Lett* 11:1839-1842. http://dx.doi.org/10.1016/S0960-894X(01)00318-3

Milligan SR *et al.* 1999. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *J Clin Endocrinol Metab* 83:2249–2252. http://dx.doi.org/10.1210/jcem.84.6.5887

Minomol M. 2005. Culture of Gold fish Carassius auratus using medicinal plants having immunostimulant characteristics [Dissertation]. MS University, India.

Monteiro PRR *et al.* 2000. Polycyclic aromatic inhibit in vitro ovarian steroidogenesis in the flounder (*Platichthys flesus* L.). *Aquat Toxicol* 48:549–559. http://dx.doi.org/10.1016/S0166-445X(99)00055-7

Obaroh IO, Achionye-Nzeth CG. 2011. Effects of crude extract of *Azadirachta indica* leaves at controlling prolific breeding in *Oreochromis niloticus* (Linnaeus 1978). *Asian Journal of Agricultural Research* 5:277-282. http://dx.doi:10.3923/ajar.2011.277.282

Ososki AL, Kenelly EJ. 2003. Phytoestrogens: a review of the present state of research. *Phytother Res* 17:845-869. http://dx.doi.org/10.1002/ptr.1364.

Pattiasina BJ *et al.* 2010. Ovarian development of female mud crab, *Scylla serrata* supplemented with cholesterol and injected with serotonin. *Jurnal Akuakultur Indonesia* 9:67-76.

Quinitio ET *et al.* 2007. Ovarian maturation stages of the mud crab *Scylla serrata*. *Aquacult Res* 38:1434–1441. http://dx.doi.org/10.1111/j.1365-2109.2007.01650.x.

Rafi MM *et al.* 2000. Modulation of bcl-2 and cytotoxicity by licochalcone-A, a novel estrogenic flavonoid. *Anticancer Res* 20:2653–2658.

Raj A *et al.* 2011. A antifertility activity of medicinal plants on reproductive system of female rat. *International Journal of Bio-Engineering Sciences and Technology-IJBEST* 2:44-50.

Sangat HM *et al.* 2000. Kamus Penyakit dan Tumbuhan Obat Indonesia (Etnofitomedika 1). Jakarta(ID): Yayasan Obor Indonesia.

Sivaram V *et al.* 2004. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. *Aquaculture* 237:9–20.

Stadtlander T *et al.* 2008. Treatment with saponins from *Trigonella foenum graecum* and *Quillaja saponaria* influences sex ratio in Nile tilapia (*Oreochromis niloticus*) larvae. In: Fitzsimmons K, Liping L (eds.) proceedings of the 8th international symposiums on tilapia aquaculture. Cairo, Egypt. pp. 355-364.

Trant JM *et al.* 2001. Developmental expression of cytochrome P450 aromatase genes (CYP19a and CYP19b) in zebrafish fry (*Danio rerio*). *J Exp Zool* 290:475–483. http://dx.doi: 10.1002/jez.1090

Tsai CL *et al.* 2000. Effects of gonadal steroids on brain serotogenic and aromatase activity during the critical period of sexual differentiation in tilapia, *Oreochromis mossambicus. J Neuroendocrinol* 49:894-898. http://dx.doi:10.1046/j.1365-2826.2000.00536.x

Venketramalingam K *et al.* 2007. *Zingiber officinalis* an herbal appetizer in the tiger shrimp *Penaeus monodon* (Fabricius) larviculture. *Aquac Nutr* 13:439–443. http://dx .doi:10.1111/j.1365-2095.2007.00495.x

Warrier SR *et al.* 2001. Occurrence of vertebrate steroids, estradiol 17ß and progesterone in the reproducing females of the mud crab *Scylla serrata*. *Comp Biochem Physiol Part A* 130:283-294. http://dx.doi:10.1016/S1095-6433(01)00385-3

Wu G *et al.* 2007. Immunological and biochemical parameters in carp (*Cyprinus carpio*) after Qompsell feed ingredients for long-term administration. *Aquac Res* 38:246–255. http://dx.doi:10.1111/j.1365-2109.2007.01660.x