# Effect of turkey berry (*Solanum torvum*) stalk extract on masculinization of guppy fish (*Poecilia reticulata*)

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**Abstract.** Turkey berry stalk contain steroid hormones. It has potential as one of the natural hormones for sex direction in fish. Sex direction aims to produce monosexed fish. Sex direction in fish is one of the genetic engineering. Guppy fish is one of the aquatic fishery commodities. The attraction of guppy fish lies in the beauty of its color, attractive and varied colors are obtained in male guppy fish. The aims of this study to determine the effect of masculinization of guppies using turkey berry stalk extract and the optimal dose on the percentage of male and female guppies. The experimental group used a Completely Randomised Design (CRD) with two factors and three replications. The first factor was is the length of time the broodstock was immersed (24 hours, 48 hours). The second factor is the dose of turkey berry stalk extract (50 ppm, 100 ppm, 200 ppm). The parameters observed included male of guppy larvae, female of guppy larvae and survival rate. The results showed that the optimal dose of turkey berry stalk extract to produce a percentage of male guppy fish in the immersion treatment with a time of 24 hours, a dose of 200 ppm of 66.47 ± 9.13%.

## 1 Introduction

Guppy fish (*Poecilia reticulata*) is one of the most popular freshwater ornamental fish that has high economic value and is an export commodity [1]. Guppies have a variety of attractive colors such as red, blue, yellow, and other colors [2]. Morphologically, the body shape of male guppies is slimmer with an attractive body pattern compared to female guppies [3]. Therefore, the demand and selling price of male guppies are relatively higher than female guppies. To produce a higher percentage of males in guppy fish farming, a sex-direction technique can be used [1], [4].

Sex reassignment technology is one technique that can be used to obtain monosex male offspring [5]. [6] states that sexing fish from male to female or vice versa can be implemented through hormonal engineering. In general, the hormones often used in fish sexing are steroid

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hormones. Steroid hormones are synthetic hormones such as 17α-methyltestosterone, estradiol-17β, and aromatase inhibitors [7]. However, the use of the hormone 17α-methyltestosterone has a negative impact on the environment, liver damage in test animals, and can even cause death. In addition, the continuous use of this hormone can leave residues and can affect food safety [8], [9]. Therefore, it is necessary to replace synthetic steroid hormones with natural steroid hormones that can be applied in masculinizing guppies.

One of the natural ingredients that can be used in masculinizing guppies is the use of turkey berry stalk extract. The turkey berry (*Solanum torvum*) is a type of aubergine plant that has properties as a traditional medicine because it contains vitamins and minerals [10]; chemical compounds such as alkaloids, flavonoids, and phenol compounds [11]; tannins, saponins, and steroids [12]. Steroid hormones in turkey berry plants are found in leaves, fruits, and stalks. However, steroid hormone levels in the stalk were higher than in the fruit and leaves [13]. Some research results regarding the utilization of turkey berry plants in aquaculture include [13] that immersion of common carp larvae using extracts of Turkey berry leaves and stalks can increase the percentage of sex by 82.22%. [14] The addition of turkey berry flour in feed can inhibit tilapia testicular cells. [15] Immersion of 1 mg $L^{-1}$ turkey berry extract produced 67% male guppies. Based on the content of turkey berry has the potential to be used as a natural steroid hormone for masculinizing guppies. The purpose of this research was to determine the effect of turkey berry stalk extract on percentage of male guppy larvae and percentage of female guppy larvae.

## 2 Material and methods

### 2.1 Materials

The materials used in this research are as follows: a) Guppy fish broodstock obtained from local fish shop in Banda Aceh and Aceh Besar; b) Turkey berry stalk extract; c) distilled; d) water; e) ethanol; f) commercial feed and artemia.

### 2.2 Research methods

This study was conducted from June to August 2023 at the Faculty of Fisheries Laboratory, Abulyatama University, Aceh Besar. Maceration of turkey berry stalk extract at the Integrated Laboratory of Abulyatama University. FTIR analysis at the Chemistry Laboratory of Syiah Kuala University and quantitative steroid testing at the Gajah Mada University Laboratory. This study used the Completely Randomised Design (CRD) method with two factors and three replications.

The first factor was the length of time the pregnant broodstock was immersed, namely 24 hours and 48 hours. The second factor was the dose of turkey berry stalk extract consisting of 50 ppm, 100 ppm, and 200 ppm. The choice of soaking time and dose of rimbang stalk extract in this study was based on the balance between the effectiveness of the compounds in the extract and the tolerance of guppies to exposure to these compounds. Commonly used soaking times are between 12 to 48 hours, with extract doses selected in the range of 50 ppm to 200 ppm to avoid toxic effects harmful to fish. The treatment combinations used are presented in Table 1.

**Table 1**. The treatment combination used

| Time | Dosage | | |
|---|---|---|---|
| | **50 ppm** | **100 ppm** | **200 ppm** |
| 24 hours | $PA_1$ | $PB_1$ | $PC_1$ |
| | $PA_2$ | $PB_2$ | $PC_2$ |
| | $PA_3$ | $PB_3$ | $PC_3$ |
| 48 hours | $PA_1$ | $PB_1$ | $PC_1$ |
| | $PA_2$ | $PB_2$ | $PC_2$ |
| | $PA_3$ | $PB_3$ | $PC_3$ |

## 2.3 Research procedure

The research was conducted in several stages, including: a) Preparation of Turkey berry stalk extract. Turkey berry stalks were washed and dried for 3 days in the sun. After drying, turkey berry stalks were extracted using the maceration method with the ratio of ingredients and solvents 1: 8 (b/v). The next step was evaporated using a Rotary Evaporator for 3 hours [6]. The turkey berry stalk extract obtained was then characterized by FTIR and steroid quantitatively; b) Preparation of fish sample. The fish sample used was 36 guppy broodstock candidates in a ratio of 1:2 (12 males and 24 females). Guppy broodstock reared in an aquarium with a volume of 72 liters. Broodstock candidates are kept separately between males and females. The feed used during spawning was commercial feed, given in *ad libitum*. Next stage, the broodstock is moved to the spawning container. The spawning process was carried out for 5 days. After spawning, the male broodstock was separated from the female broodstock. The broodstock that showed pregnant characteristics were transferred to a plastic container with a capacity of 15 liters. After 12 days post-spawning, pregnant broodstock were soaked with turkey berry stalk extract for 24 hours and 48 hours at doses according to each treatment. After the soaking was complete, the broodstock were kept in another container until they gave birth. c) Larval rearing. Larval rearing was conducted for 60 days. The rearing process is carried out until the larvae can show morphological characteristics and can be calculated. Male guppies have a morphology with a relatively slender body shape and beautiful patterns and fins while female guppies have a large body size compared to male guppies [4]. During the rearing period, the larvae were fed with artemia, the frequency of feeding was three times a day at 08.00, 13.00 and 17.00 WIB.

## 2.4 Research parameters

### *2.4.1 Male guppy larvae*

The percentage of male guppy larvae is calculated based on the formula [4]:

$$\% \text{ male} = \frac{\textit{Number of males}}{\textit{Number of total}} \times 100\% \quad (1)$$

### *2.4.2 Female guppy larvae*

The percentage of male guppy larvae is calculated based on the formula [4]:

$$\% \text{ female} = \frac{\textbf{\textit{Number of female}}}{\textbf{\textit{Number of total}}} \times 100\% \quad (2)$$

### *2.4.3 Survival rate*

The value of the percentage survival rate of guppy larvae is calculated based on the formula [16]

$$SR = \frac{\textbf{\textit{Nt}}}{\textbf{\textit{No}}} \times 100\% \quad (3)$$

Where: SR = Survival rate (%); Nt = Number of fish at the end of maintenance (fish); No = Number of fish at the beginning of maintenance (fish).

### 2.5 Data analysis

The data is analyzed using Analyze of Variance (ANOVA) in SPSS 27 software to determine the effect of turkey berry stalk extract for the treatment given. If there are significant results, the calculation is continued with the least significance difference test.

## 3 Result and discussion

The results of the Fourier Transform Infra-Red (FTIR) spectroscopy test of turkey berry stalk extract are presented in Figure 1.

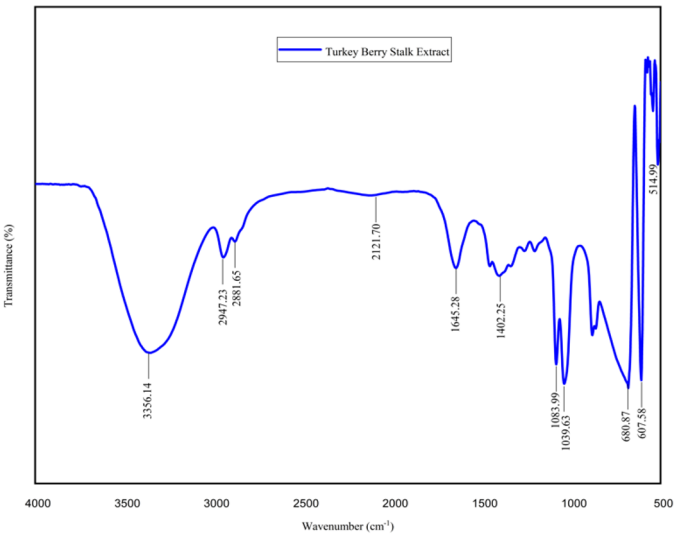


**Fig. 1.** FTIR test result of turkey berry stalk extract

Based on Figure 1 shown that the isolation of β-steroid compounds obtained from turkey berry stalks is in the form of white needle crystals. The results of IR spectroscopic analysis showed absorption bands at wave numbers 2947.23 -2881.65 $cm^{-1}$ indicating the presence of

saturated C-H aldehyde groups. The O-H strain gave a strong absorption band at a wave frequency of 3356.14 $cm^{-1}$ and identified that the reduced absorption peak, the unconjugated C=C strain at 1645.28 $cm^{-1}$, nitromethane N=O at 1402.25 $cm^{-1}$, at a frequency of 1083.99-1039.63 $cm^{-1}$ there was a C-O alcohol group [17].

The combination of immersion dose and duration had a significant effect on survival, male and female sex percentage of guppies. This indicates that the two factors (time and doses) interact with each other in influencing the results. Based on the results of the ANOVA, masculinization of Guppy fish using Turkey berry (*Solanum torvum*) stalk extract through immersion of pregnant broodstock has a significant (P>0.05) effect on the percentage of male guppy larvae, the percentage of female guppy larvae and the survival rate of guppy larvae (Table 1).

**Table 2**. The results of measuring research parameters of guppy larvae for 60 days.

| Time | Dosage (ppm) | Research Parameters | | |
|---|---|---|---|---|
| | | Survival rate (%) | Male of guppy larvae (%) | Female of guppy larvae (%) |
| 24 | 50 | $91,91 \pm 7,01^{a}$ | $52,63 \pm 10,98^{b}$ | $47,37 \pm 10,98^{bc}$ |
| | 100 | $97,43 \pm 4,43^{b}$ | $51,82 \pm 10,25^{b}$ | $48,17 \pm 10,25^{bc}$ |
| | 200 | $95,55 \pm 7,69^{ab}$ | $66,47 \pm 9,13^{d}$ | $33,52 \pm 9,13^{a}$ |
| 48 | 50 | $100 \pm 0,00^{c}$ | $55,68 \pm 10,01^{bc}$ | $44,31 \pm 10,01^{b}$ |
| | 100 | $92,59 \pm 8,48^{a}$ | $64,76 \pm 8,54^{c}$ | $35,23 \pm 8,54^{a}$ |
| | 200 | $95,83 \pm 7,21^{ab}$ | $45,53 \pm 2,32^{a}$ | $54,47 \pm 2,32^{c}$ |

Note: Different superscript letters in the same row show significant differences (p<0.05).

After 60 days of maintenance of guppy fish larvae, the highest survival rate was obtained at the time of 48 hours of immersion of pregnant broodstock (dose of 50 ppm) of 100 ± 0.00%. The high survival rate in these treatments is thought to be due to the chemical content in the turkey berry stalk extract that can function as an antioxidant so as to protect the tissue from the influence of free radicals. Similarly, the immersion treatment for 24 hours with a dose of 100 ppm has a survival rate of 97.43 ± 4.43%. This shows that turkey berry stalk extract contains bioactive compounds that have a positive effect on guppy fish. In addition, the dose is in a low concentration range so that it does not cause physiological disturbances of guppies, a relatively short exposure time of turkey berry stalk extract, the ability of guppies to adapt and the ability of guppies to adsorb chemical compounds of turkey berry stalk extract so that the survival rate in each treatment tends to be high. On the other hand, the feed factor also affects the survival rate of Guppy larvae. [18], stated that important factors affecting the growth and survival of guppies are the availability of food and environmental quality. One feed that plays an important role in increasing growth and survival is artemia [19]. In this study, the natural food given to guppy larvae is artemia. Therefore, the need for food for larvae is fulfilled in terms of quantity and quality it affects the survival rate.

The percentage of male Guppy fish larvae found in the 24-hour immersion treatment (200 ppm dose) amounted to 66.47 ± 9.13%. While the percentage of female Guppy fish larvae found in the 48-hour immersion treatment (200 ppm dose) amounted to 54.47 ± 2.32%. The high percentage of male Guppy larvae in the treatment is thought to be that the steroid hormone content in turkey berry stalk extract is able to affect the sex formation of guppy larvae. Turkey berry which comes from the Solanaceae family, presents a steroid group as solasodine ($C_{27}H_{43}O_2N_7$) which is an important source for the synthesis of steroid hormones [20]. Based on the test results of turkey berry stalk extract conducted at the Integrated Research and Testing Laboratory of Gadjah Mada University, turkey berry stalk contains 704.85 µg/mL steroid hormones. According to [21], it states that steroids are useful for helping testosterone to trigger sex reversal.

The higher the dose of turkey berry stalk extract in the 24-hour immersion treatment of pregnant broodstock, the higher the percentage of male guppy larvae. However, in the 48-hour immersion treatment of pregnant broodstock, the higher the concentration of turkey berry stalk extract, the percentage of male guppy larvae tended to decrease. This phenomenon indicates that the correct dosage, trigger material and duration of treatment are important factors in increasing the sex ratio of the resulting larvae. This is in line with several studies, including research [15] which used a dose of eggplant fruit as much as 1 mg/L to produce a percentage of male guppy puppies as much as 67%. The use of hybrid coconut water at a dose of 40% gave the highest male ratio, which was 96.90% [22] and 50% dose also gave good results with a percentage of males of 96.90 ± 1.34% [23]. Similarly, the length of time for soaking using coconut water shows that soaking for 12 hours can increase the percentage of male sex puppies, with the highest results at a dose of 60% coconut water [22].

## 4 Conclusion

The use of turkey berry stalk extract has the potential to masculinize Guppy fish. Guppy masculinization using turkey berry stalk extract through immersion of pregnant broodstock has a significant ($P<0.05$) effect on the percentage of male guppy larvae, a percentage of female guppy larvae and a survival of guppy larvae. Immersion treatment with a time of 24 hours, a dose of 200 ppm produced a percentage of male Guppy fish larvae of 66.47 ± 9.13%, the percentage of female Guppy fish larvae 33.52 ± 9.13% and the survival rate 95.55 ± 7.69%.

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