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Effects of *Tribulus terrestris* and *Basella alba* Extract on Sex Reversal on *Poecilia reticulata*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study examined the effects of *Tribulus terrestris* and *Basella alba* on sex reversal in the guppy, *Poecilia reticulata*. This study aimed to introduce a new environmentally friendly method for masculinization in *P. reticulata*. Since male guppy has higher commercial value than females. *T. Terrestris* (ET) and *Basella alba* (EB) are a natural, non-toxic herb that helps enhance testosterone levels in humans and animals. Different concentrations (0.0, 5.0, 10.0, and 15.0 g L-1) of ET and EB were investigated for sex reversal in *P. reticulata*. The plant extracts were administered by immersion of newly hatched offspring once a week for two months. The dosage of 15.0, g L-1 ET was the most effective dosage that ensured a maximum male ratio (82.0, $p<0.01$). Although, sex ratios of fish in treatment 5.0 and 10.0 g L-1 ET were not significantly different from the expected 1:1 ratio, in these two groups treatment with ET and EB also resulted in a higher number of males (71.9 and 61.1; 82.0 and 78.5, respectively), than control ($p>0.05$). Total survival rates in all treatments and control were uniformly high ranging from 92% and 97% ($p>0.05$). It is concluded that ET has no negative effect on the survival rate of *P. reticulata*. All groups of ET-

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treated fish exhibited growth acceleration compared to the control group, but only ET treatment at the concentration of 15 and 10 g L-1 ET significantly improved growth rate of *P. reticulata* ($p<0.01$). Histological examinations revealed that the testes of fish treated with ET and EB extract contained all stages of spermatogenesis. Sex reversal in *P. reticulata* demonstrated that ET and EB-treated progenies showed successful sex reversal, spermatogenesis, and better growth rate than untreated progenies.

Keywords: Basella alba; Tribulus terrestris; Poecilia reticulata; sex reversal; spermatogenesis.

1. INTRODUCTION

Aquaculture is the technique of breeding, cultivating, and harvesting fish, shellfish, algae, and other organisms in a variety of aquatic environments. Ornamental fish production is an important component of the aquaculture industry that is both an attractive addition to your aesthetic design and a source of employment [1]. "*Tribulus terrestris* L. (ET) is an herb native to China, Japan, Korea, and Western Asia, as well as the southern parts of Europe and Africa. It has been shown to enhance testosterone levels safely and organically. Human and animal ET (*T. terrestris* L.) administration boosts athletic performance" [2,3,4]. "Testosterone is a sex hormone that has a clear role in sexuality. At physiological level of testosterone in the body causes the larvae's embryonic differentiation into male and subsequent growth along this line. Testosterone is the biosynthetic precursor to both androgens and estrogens in teleosts" [5,6]. "However, 11-oxygenated androgens are commonly found in high concentrations in male fish plasma and tissues and are largely regarded as the most important physiological androgens in males" [7].

"There are substantial differences in the efficacy of various phytochemicals for developing all-male fish populations, and the potential for analyzing and virilizing the effects of such plant extracts must be properly demonstrated. It has been proven that *T.terresteris* increases testosterone levels" [2,3]. "*T. terrestris* contains many chemicals known as steroidal saponins. The saponin identified in *T. terrestris* that is thought to be responsible for its effect on testosterone levels is protodioscin" [8-10].

"Other herb with potentially sex-reversing effects, is *Basella alba* (EB), a fast-growing vegetable likely originating in India" [11]. "During *in vitro* investigations, aqueous and methanol extracts from the dry leaves of *Basella alba*, it was found to contain active components that increased testosterone synthesis in adult male rat testes" [12,13]. "This edible plant has also been characterized as having nutritional qualities, including androgenic, in numerous cultures' traditional treatments" [14].

Poecilia reticulata, or live-bearing guppy, is a popular aquarium fish that aquarists use to develop new color variants. Because males in this species are more desirable than females, business interest in preserving and breeding male populations has grown significantly. *P.reticulata* can be masculinized using a simple and effective synthetic hormone therapy [15,16,17]. Synthetic hormones, on the other hand, are more expensive than plant extracts, and their fate in water and sediment informs us about the possible risks of using synthetic hormones [18]. Plant extracts could be employed as an alternate method for commercializing all-male products.

The current study's purpose is to investigate the effects of *T. terrestris* and *B. alba* on *P. reticulata* sex reversal. Despite their multiple biological uses in traditional medicine, there is a scarcity of research on the influence of *T. terrestris* and *B. alba* on guppy sex reversal and growth parameters. Furthermore, considering the increasing use of plant extracts as a potential replacement for synthetic hormones and chemotherapeutics, scientific research into the effects of these two plant extracts in guppy culture is essential.

2. MATERIALS AND METHODS

2.1 Plant Extracts Preparation

T. terresteris and *B. alba* extracts were prepared at Kongunadu Arts and Science College (Coimbatore. Tamil Nadu, India). *T. terresteris* and *B. alba* aqueous extracts were prepared by steeping 18 g of ET and EB pure and fine extract in 1500 mL distilled water for 30 minutes and then filtering it twice using Whatman filter paper [17,19]. This solution was prepared eight times (weekly for 60 days, for 20 L aquariums), yielding

three duplicates for the ET and EB treatments (18 g of ET and EB were used per immersion, and the larvae were exposed eight times). Phytochemical analysis of plant extract was carried out using Abdullahi et al. [20] procedure.

2.2 Maintenance of *Poecilia reticulate*

A guppy stock (90 fish) was collected from an aquarium the Male's mean weight and length were 0.05 g and 2.16 cm (mean±SE), respectively, and female's mean weight and length were 0.05 g and 2.19 cm (mean±SE). Seven males and seven females were stocked in an aquarium with re-circulating water (26ºC) and a 12:12 h light/dark cycle. At least ten times every day, the broodstock aquaria were checked for offspring (especially in the early morning). The newly hatched fries were immediately removed from the aquarium, numbered, measured, and placed in 12 miniature glass aquariums, each containing 20L of water, and aerated with a 4 cm air stone, all the fry used in this experiment were the same age.

2.3 Treatment of *P. reticulate* Fry with *T. Terrestris* and *B. alba* Plant Extracts

Fish were randomized and divided into one of four treatment groups, and each received one of the four *T. terresteris* and *B. alba* concentrations formulated: 0 (Control), 5.0, 10.0, and 15 gL-1. The treatment began at 0 days post-hatching (dph) and lasted until 60 (dph). The four treatments were carried out concurrently, each with three replicates. The aquarium system was a static bath that needed to be changed manually. Each aquarium is stacked with 35 fry. Thus, for the experiment, 12 batches, including a control batch, were used, giving a total of 420 fry. The effect of ET and EB on the growth and survival rate of *P. reticulate* was measured, and images of the testis structure were captured using an inverted trinocular microscope (Olympus model).

2.4 Sexing of Fish

At the end of the experiment (60 dph), 20 juvenile fish from each of the 8 groups were sexed using the conventional acetocarmine squash gonad procedure [21].

2.5 Determination of Physicochemical Parameters

Temperature, pH, and oxygen were measured every 15 days. The dissolved oxygen (DO) was measured weekly using the Azide modification of Winglers (1888) [22]. The temperature was recorded with a thermometer (Broken brand) scale - 20 to 110°C with an accuracy of ±1 digit; pH was determined with a potentiometer Corning brand with an accuracy of ±1 digit.

2.6 Statistical Analysis

A Kruskal-Wallis one-way analysis of variance by ranks (SPSS 10.0 for Windows) was used to examine differences in mortality with body weight and length between groups, followed by the Duncan non-parametric multiple comparison approach. The chi-square (X2) test was used to compare differences in offspring sex ratios based on secondary sex characteristics (in males: gonidium, veil-like caudal fin, greater body pigmentation, and small body size) and gonad histology [23].

Table 1. Phyto-chemical analysis of *Basella alba* and *Tribulus terrestris*

Phyto-chemical analysis	Plant	
	Tribulus terrestris	***Basella alba***
Solvent for extraction	Ethanol extract	Ethanol extract
Tanin	+	+
Saponin	+	+
Alkaloid	+	+
Carbohydrate	-	-
Glycoside	-	-
Flavonoid	-	-
Steroid/Terpenoid	-	+

3. RESULTS AND DISCUSSION

Qualitative analysis for phytochemicals revealed the presence of tannins, alkaloids, saponin, and steroids in both *Basella* and *Tribulus* water extracts while flavonoids, glycosides, and carbohydrates were not present in any of the extracts (Table 1).

3.1 Gonadal Differentiation

Sexing of fish was done by the standard acetocarmine squash technique of gonads. Histological and microscopic studies of the gonads were also performed (Fig. 1).

3.2 Effect of *T. terrestris and B. alba* on the Sex Ratio of *P. reticulata*

In the present work, we achieved 80 % masculinization by immersing 0-day-old fry for 60 days in water containing 15 g ET per litter. All groups of ET and EB- and EB-treated fish exhibited more males than females compared to the control group. The concentration of 15 g L^{-1} significantly altered sex ratio in P. *reticulate* ($p<0.001$; Table 2). Dietary treatment with methanol extract of *B. alba* was found to enhance the percentage of males in the guppy *P. reticulata* [24]. Several pathways have been proposed to be related to the functional processes of phyto-compounds that cause both masculinization and feminization at various concentrations [25]. In the first series of tests (control groups), the sex ratio was 1:1 (male: female) (Table, 1). A similar high yield from *B. alba* leaves was reported using methanol in a series of extractions with petroleum ether, ethyl acetate, and methanol [24].

In the second series of tests (0.05 g L-1 ET-treatment groups), the sex ratio was 53:38 (male: female. There were no inter-sex fish recorded in this study. In traditional medicine, *B. alba* has been used to cure sexual asthenia and infertility in men [26]. Its leaves were observed to promote testosterone production in testicular fractions and Leydig cell cultures, as well as in normal adult albino male rats [12,27]. A similar increase in serum testosterone level was seen in male rats administered with *B. alba* aqueous extract by stomach intubation [12].

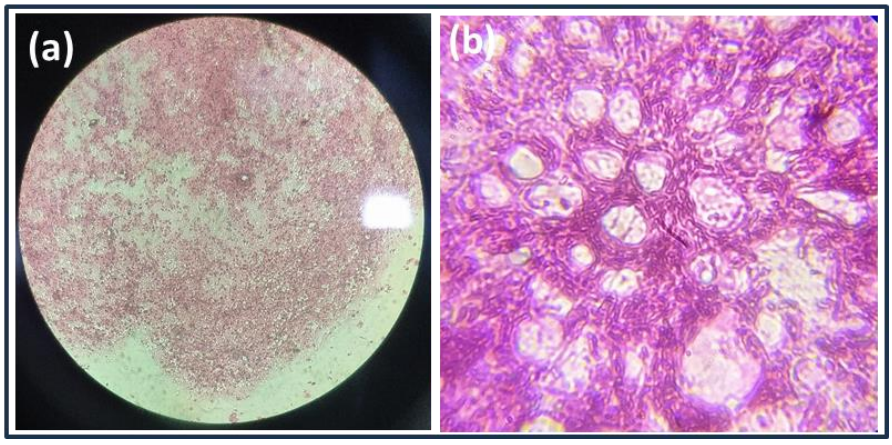

Fig. 1. Cross-section of Guppy gonad (a) Testis, (b) Ovary. Olympus microscope- 20x

Table 2. Effect of ET and EB on the sex ratio of *Poecilia reticulate*

Experiment	Dose ($g\ L^{-1}$)	Treatment duration (day)	Sex ratio Male: Female ♂ : ♀	Sex ratio (%) Male: Female ♂ : ♀
ET	0 g/kg	60	9: 11	45: 55
	5 g/ kg	60	13: 2	86.6**:** 13.3
	10 g/kg	60	12: 3	80**:** 20
	15 g/kg	60	14: 1	93.3**:** 6.66
EB	0 g/kg	60	9: 11	45: 55
	5 g/kg	60	11: 4	73: 26.6
	10 g/kg	60	10: 5	66.6**:** 33.3
	15 g/kg	60	13: 2	86.6**:** 13.3

Table 3. Survival and growth rate at the end of the experiment

Treatment	Dose ($g\ L^{-1}$)	Survival rate (%)	Total length (cm)	Body weight (g)
ET	0 g/kg	92 %	2.16±0.04	0.05±0.003
	5 g/ kg	97 %	2.06±0.04	0.04±0.00
	10 g/kg	95 %	2.23±0.02	0.05±0.00
	15 g/kg	92 %	2.19±0.02	0.05±0.001
EB	0 g/kg	91 %	2.17±0.05	0.05±0.00
	5 g/kg	92 %	2.20±0.06	0.04±0.00
	10 g/kg	90 %	2.24±0.02	0.05±0.00
	15 g/kg	89 %	2.23±0.04	0.05±0.001

Table 4. Physico-chemical analysis at the end of the experiment

Treatment	Temperature (ºC) (n=20)	pH (n=20)	Dissolved Oxygen (DO) (n=20)
Control	25.9±0.5	7.00±0.01	5.25±0.03
EB1	26.01±0.03	6.89±0.00	4.68±0.00
EB2	26.05±0.04	6.85±0.34	4.50±0.09
EB3	26.09±0.03	6.33±0.30	4.36±0.01
ET1	27.01±0.03	6.83±0.03	4.02±0.01
ET2	27.03±0.03	6.81±0.02	4.01±0.01
ET3	27.06±0.02	6.83±0.03	4.02±0.04

3.3 Survival and Growth of the *Tribulus terrestris* and *-Basella alba* Administered Fish

The survival and growth rates in total length and body weight of *P.reticulata.* are shown in Table 4. Total survival rates in all treatments and controls were uniformly ranging from 83 to 87 % ($p>0.05$). All groups of ET-treated fish exhibited successful growth acceleration compared to the control group and EB. Still, ET treatment at the concentrations of 10 and 5 g L^{-1} ET group was significantly higher compared to the control group. The survival rate after 28 and 60 days of experimentation was not affected by any of the therapies investigated when compared to the control. These data demonstrated that the therapies utilized had no significant harmful effect on the tested fry.

3.4 Water Quality Analysis

Temperature varied between control 25.9; EB1 26.01; EB2 26.05; EB3 26.09 and ET1 27.01; ET2 27.03; ET3 27.06°C. The pH varied from Control 7.00; EB1 6.89; EB2 6.85; EB3 6.33 and ET1 6.83; ET2 6.81; ET3 6.83. the concentration of oxygen varied from control 5.25; EB1 4.68; EB2 4.50; EB3 4.36 and ET1 4.02; ET2 4.01; ET3 4.02 (Tab 2). The ideal temperature for keeping them is between 20 and 30 degrees Celsius, with a minimum of 18 degrees Celsius. According to Axelrod [28], the ideal temperature is 25.5°C. The temperature affects growth and gonadal maturation [29]. The temperature in this work was 28°C [30]. The pH values obtained in this study varied from 8.1 to 8.8, which is within the ideal range for this species' culture.

4. CONCLUSION

The findings of this study suggest that these plants could be used as an alternate strategy to develop entirely male Guppy populations in an environmentally sustainable way utilizing a natural product. Comparing two plants *T. terrestris*, could be considered more potent for inducing masculinization than *B. alba* in the Guppy because it resulted in a higher male percentage. However, the highest percentage of males produced by plant extracts was discovered to be significantly lower than the optimal criterion of a 100% male population. Thus, more research is needed to develop an appropriate treatment regimen for producing all-male guppy populations using plant extracts and to offer solid evidence of their efficiency as a sex-reversal agent in guppy culture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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