



Assessment *Tribulus terrestris* on sex-reverse on Nile Tilapia with respect to its chemical composition

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Abstract: *Tribulus terrestris* considered the save feed additive for sex reverse of Nile tilapia offspring. Monosex fries of Nile tilapia (mean weight 0.020 ± 0.005 g; mean length 1.20 ± 0.003 cm) were subjected to dietary treatment (commercial fish feed, 32% crude protein) with Aquatic extract of *Tribulus Terrestris* at different concentrations (0.0, 1.0, 2, 3 g/kg feed were orally administered to sexually undifferentiated fries from the 3rd to the 28th days post hatching to produce male tilapia population. to evaluate the efficacy of the plant extract for induction of sex reverse and growth in fish. Dietary supplementation with the plant extract at the concentration of 2.0 g/kg feed showed the highest percentage (97.43 ± 0.13) of males and may be considered to be the best concentration among the selected concentrations for monosex tilapia production with *Tribulus Terrestris* extract. The result of the study indicates that aquas extract of *Tribulus Terrestris* extract may have potential for inducing sex reversal in fish and this biodegradable natural plant material may be used for monosex tilapia production instead of synthetic steroids.

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Keywords: *Tribulus terrestris*, Monosex, fries, Nile tilapia, sex-reversal

1. Introduction

In recent year, herbal supplements have been tested in aquaculture as an alternative to chemicals. Using of plant-based additives in aquaculture is one of the methods used to improve weight gain and feed efficiency in cultured fish (Dada, 2015). Plants are natural source of safer and cheaper chemicals. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions (Citarasu, 2010). Herbal supplements are efficient and more environmentally friendly in disease management that enhances immunity and resistance to pathogens (Hussain et al., 2009) *Tribulus terrestris* L. (puncture vine) has been dubbed Nature's Viagra is one of the most famous medicinal herbs used for sexual reverse in cultured tilapia used to improve physical performance, androgenic in males and estrogenic in female (increased various sexual hormones: including testosterone, dehydroepiandrosterone (DHEA), luteinizing hormone (LH), estradiol, follicle-stimulating hormone (FSH) and estrogen hormone). In deed, the pharmacological activities and clinical trials of natural products from *Tribulus species* have been found as antioxidant. Pharmaceutical preparations and

dietary supplements based on the phenolic fraction of *Tribulus Terrestris* are on nutrient supplement and muscle tone (Turn and Cek, 2007). Are now commercially marketed under various names, such *Tribulus Terrestris* capsules® (Adiakan et al., 2000). Phenolic compounds are a group of chemical compounds that are widely distributed in nature. Phenolic compounds are reported to have multiple biological effects including antioxidant activity (Kavitha and Subramanian, 2011). In foods, phenolic may contribute to the oxidative stability of products. In addition, health-protecting capacities of some and anti nutritional properties of other plant phenolics are of great importance to producers (Gültepe et al., 2014). The extensive chemical diversity in substances having potential biological significance to be found in *Tribulus* plants including steroidal saponins, flavonoids, alkaloids, phenolic compounds and phytosterols that have been isolated. The antioxidative properties of *tribulus terrestris* growing were studied. A combination of experimental and statistical methods were applied to study and to compare the radical scavenging activity and the structure of eleven flavonoid components. Quantitative HPTLC studies include estimation of diosgenin. *Tribulus Terrestris* was found to contain 0.08% of dysgenic (Gülçin et al., 2007).

The objective of present study was inducing sex reverse and growth performance of Nile tilapia. Current study was conducted to evaluate the effect of *Tribulus Terrestris* extract on sex reverse and growth performance and immune stimulant on cultured Nile tilapia.

2. Materials and Methods

Plant materials

Fruits, leaves and roots of *Tribulus Terrestris* used in this study were collected at the end of November 2012 from Kafr El Sheikh governorate, Egypt (Fig.1.)



Fig., 1: showing *Tribulus Terrestris* plant.

Preparation of aqueous extracts:

The air dried fine powdered plant fruits, leaves and roots (500 g) were infused in distilled water until complete exhaustion. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuum and dried using a rotary evaporator at 60 °C (Kandil *et al.*, 1994). The final dried samples were stored in labeled sterile bottles and kept at -20 °C.

The aqueous extracts of *Tribulus Terrestris* were prepared by boiling 500 g pure and fine extract of *Tribulus Terrestris* in 1500 mL distilled water for 30 min and then filtering it with a whatman filter paper twice (Gauthaman and Adaikan, 2005). This solution was prepared 8 times (weekly for 60 days, each aquaria was 20 L), in another words, 3 replicates for the TT treatment 18 g of TT was used per immersion and the larvae were exposed 8 times.

Determination of antioxidant activity:-

a) Radical scavenging activity using DPPH assay

Antioxidant activity was also determined by DPPH assay using spectrophotometer at 517 nm. **Gülçin *et al.*, 2007**. Extract of different concentrations (50, 100, 200 and 400 µg/ml, respectively) and TBHQ (50, 100, 200 and 400 µg/ml) were taken in different test tubes. Four milliliter of 0.1 mM methanol solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as the same without any extract sample and MeOH. The

changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = [(AB-AA)/AB] \times 100$$

Where: AB: absorption of blank sample (t=0 min), AA: absorption of sample solution (t=30 min).

b) β-Carotene-linoleate scavenging assay

The antioxidant activity of the extract was evaluated using β-carotene-linoleate model system. β-carotene (0.1 mg) in 0.2 mL of chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 were mixed. The solvent was removed at 40 °C under vacuum and the resulting mixture was diluted with 10 mL of water and was mixed well. To this mixture, 20 mL of oxygenated water was added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 200 µL of each oil (50, 100, 200 and 400 µg/ml) and TBHQ (50, 100, 200 and 400 µg/ml) in ethanol. TBHQ was used for comparative purposes. A control containing 200 µL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath and the absorbance at 470 nm was taken at zero time (t= 0). The absorbance was continued to be measured until the colour of beta-carotene disappeared in the control tubes (t =60 min) at an interval of 15 min. **Gülçin (2006)**. A mixture prepared as mentioned above without carotene served as blank. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extract

was evaluated in terms of bleaching of the carotene using the following formula,

$$\% \text{ Inhibition} = [(AB-AA)/AB] \times 100$$

Where: AB: absorption of blank sample (t=0 min).

AA: absorption of sample solution (t=60 min).

The results were expressed in % basis in preventing bleaching of β -carotene.

Identification of volatiles compounds

Gas chromatographic-mass spectrometric analysis (GC/MS)

The analysis was carried out by using a coupled gas chromatography Hewlett-Packard model (5890) / mass spectrometry Hewlett-Packard-MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The isolated peaks were identified by matching with data from the library of mass spectra (National

Institute of Standard and Technology) and compared with those of authentic compounds and published data. The quantitative determination was carried out based on peak area integration. Identification of the GC components also was confirmed with NIST mass spectra library data Adams (1995).

3. Results

Antioxidant activity *in vitro*:

The results as shown in table 1 that obtained by DPPH free radical scavenging assay of *Tribulus terrestris* volatiles was (93.51% \pm 3.1). while, the standard *Tert*-butyl hydroquinone (TBHQ) (99.33% \pm 3.1) at 400 μ g/ml.

The results in table 2 that extract was (95.69% \pm 2.2), while, the standard *Tert*-butyl hydroquinone (TBHQ) (99.38% \pm 3.5) at 400 μ g/ml.

Table 1. *In vitro* antioxidant activity (A.A) of *Tribulus terrestris* aqueous extract determined by DPPH free radical scavenging method.

	% inhibition at different concentrations of <i>Tribulus terrestris</i>			
	50(μ g/ml)	100(μ g/ml)	200 (μ g/ml)	400(μ g/ml)
water extract	75.63 \pm 1.5	84.51 \pm 2.5	90.66 \pm 2.7	96.11 \pm 3.4
♦TBHQ	77.11 \pm 2.6	83.96 \pm 2.5	97.11 \pm 3.4	99.33 \pm 3.1

♦TBHQ: *Tert*-butyl hydroquinone, standard synthetic antioxidant.

Each value represents the mean \pm S.E (Standard Error) and mean of three replicates.

Table 2. *In vitro* antioxidant activity (A.A) of *Tribulus terrestris* aqueous extract determined by β - carotene method.

	% inhibition at different concentrations of <i>Tribulus terrestris</i>			
	50(μ g/ml)	100 (μ g/ml)	200(μ g/ml)	400(μ g/ml)
water extract	76.0 \pm 2.3	85.2 \pm 2.1	92.24 \pm 3.6	97.35 \pm 4.0
♦TBHQ	76.2 \pm 2.1	86.1 \pm 2.3	94.82 \pm 3.3	99.38 \pm 3.5

♦TBHQ: *Tert*-butyl hydroquinone, standard synthetic antioxidant.

Each value represents the mean \pm S.E (Standard Error) and mean of three replicates.

Table 3. *In vitro* antioxidant activity (A.A) of *Tribulus terrestris* volatiles determined by DPPH free radical scavenging method.

	% inhibition at different concentrations of <i>Tribulus terrestris</i>			
	50(μ g/ml)	100(μ g/ml)	200(μ g/ml)	400(μ g/ml)
Volatile extract	76.13 \pm 1.1	81.49 \pm 2.4	88.81 \pm 2.6	93.51 \pm 3.1
*TBHQ	77.11 \pm 2.6	83.96 \pm 2.5	97.11 \pm 3.4	99.33 \pm 3.1

*TBHQ: *Tert*-butyl hydroquinone, standard synthetic antioxidant.

Each value represents the mean \pm S.E (Standard Error) and mean of three replicates.

Table 4. *In vitro* antioxidant activity (A.A) of *Tribulus terrestris* volatiles determined by β - carotene method.

	% inhibition at different concentrations of <i>Tribulus terrestris</i>			
	50(μ g/ml)	100 (μ g/ml)	200(μ g/ml)	400(μ g/ml)
Volatile extract	74.0 \pm 2.1	82.2 \pm 2.6	91.1 \pm 3.1	95.69 \pm 2.2
*TBHQ	76.2 \pm 2.1	86.1 \pm 2.3	94.82 \pm 3.3	99.38 \pm 3.5

*TBHQ: *Tert*-butyl hydroquinone, standard synthetic antioxidant.

Each value represents the mean \pm S.E (Standard Error) and mean of three replicates.

Table 5. Chemical composition of Aroma volatiles of *Tribulus terrestris*

No. of Peak	RT	Area%	Identified compounds
1	26.38	11.06	4-Methyl-2,6-di-tert-butylphenol
2	36.65	15.94	Hexadecanoicacid, ethyl ester
3	38.87	8.02	Phytol
4	39.72	4.40	Ethyl linoleate

Table 6. Chemical composition of Aroma volatiles of *Tribulus terrestris*

No. of Peak	RT	Area%	Identified compounds
1	7.48	0.31	2-Phenyl-1-p-tolyethanol
2	10.22	0.63	3-methyl Phenol
3	21.63	7.03	5-methyl-2-(1-methylethyl) Phenol
4	22.03	0.72	2-Methoxy-4-vinylphenol
5	23.87	4.15	2methoxy3(2propenyl) Phenol
6	35.63	0.31	2-Phenyl-1,3-dioxan-5-octadecatrienoate

Table.7. Effect of orally-administered *Terriblusterristes* aquaus extract on growth and survival rate of Nile tilapia, *Oreochromis niloticus*, after 15 days of treatment.

Treatment category	Growth parameters			Survival rate (%)		
	Mean length (cm)			Mean weight (gm)		
	Min	Max	Avg \pm SD	Min	Max	Avg \pm SD
Control	1.20	1.80	1.42 \pm 0.200	0.40	0.80	0.500 \pm 0.440 88
1.0gm/kg of diet	2.70	3.70	2.94 \pm 0.377	0.50	1.50	0.975 \pm 0.359 79
2.0 gm/kg of diet	3.50	4.20	3.80 \pm 0.221	1.80	2.30	1.970 \pm 0.154a 93
3.0 gm/kg of diet	3.10	2.20	2.66 \pm 0.341	0.40	1.30	0.754 \pm 0.350 65

a Significantly different compared to the control group ($p < 0.05$).

Table.8. Effect of orally-administered *terriblusterristes* on growth and survival rate of Nile tilapia, *Oreochromis niloticus*, after 35 days of treatment.

Treatment category	Growth parameters			Survival rate (%)		
	Mean length (cm)			Mean weight (gm)		
	Min	Max	Avg \pm SD	Min	Max	Avg \pm SD
Control	3.00	3.80	3.45 \pm 0.332	1.50	2.00	1.766 \pm 0.158 92
1.0gm/kg of diet	4.80	3.90	4.28 \pm 0.297	2.70	3.30	3.060 \pm 0.183 90
2.0 gm/kg of diet	5.60	4.30	5.06 \pm 0.396	3.20	3.90	3.500 \pm 0.269a 94
3.0 gm/kg of diet	3.30	4.20	3.79 \pm 0.262	2.50	3.20	2.810 \pm 0.199 60

a Significantly different compared to the control group ($p < 0.05$).

Table 9 Effect of orally-administered *Terriblusterristes* on growth and survival rate of Nile tilapia, *Oreochromis niloticus*, after 35 days of treatment.

Concentrations	Growth parameters		Survival rate (%) after 3 months			
	Sex reverse	Survival rate (%)	Mean weight (gm)			
			Min	Max	Avg \pm SD	
Control	43.67 \pm 2.75	72 \pm 3.21 a	130	180	157.50 \pm 22.17 100	
1.0gm/kg of diet	94.33 \pm 0.23	75 \pm 4.58a	180	200	191.25 \pm 8.53 100	
2.0 gm/kg of diet	97.43 \pm 0.13	80 \pm 2.89a	200	230	215.00 \pm 12.90a 100	
3.0 gm/kg of diet	95.27 \pm 0.62	77 \pm 3.22a	195	210	203.75 \pm 7.50 95	

Terriblus. terrestris aqueous extract: 1.0 gm/kg,2.0 gm/kg,3.0 gm/kg,

4. Discussion

The results obtained by DPPH free radical scavenging assay of *Tribulus terrestris* volatiles indicated that antioxidant activity caused an excellent

antioxidant activity towards DPPH free radicals (93.51% \pm 3.1) when comparing with the standard *Tert*-butyl hydroquinone (TBHQ) (99.33% \pm 3.1) at 400 μ g/ml). while, indicated that extract had a strong

antioxidant activity towards free radicals ($95.69\% \pm 2.2$), when comparing with the standard *Tert*-butyl hydroquinone (TBHQ) ($99.38\% \pm 3.5$) at 400 $\mu\text{g/ml}$. These results agree with that recorded by **Gülçin et al., 2007**.

Concerning the radical scavenging activity of the extract on β -carotene/linoleic acid and DPPH free radicals increased with increasing concentration of extract. These results agree with that recorded by **Gülçin, 2006**.

Regarding the treatment with 2g/kg after 3 months showed significantly ($P < 0.05$) higher (80 ± 0.00) survival percentage and sex reverse (97.43 ± 0.13) compared to control (72 ± 3.21) survival percentage and sex reverse (43.67 ± 2.72). these results agree with that recorded by **Gültepe et al., (2014)**. The results indicated that the plant extract might have no adverse effect on fish health rather it might have improved the fish survival. Similar 100% survival was observed in fed diets supplemented with 1 and 2 g/kg powdered *Tribulus Terrestris* (**Yeganeh et al., 2017**). while sex reverse with 17α methyltestosterone hormone cause 20% mortalities and sex reverse less than 80% in fries as recorded by **Griessy and El-Gamal (2012)**. Dietary supplementation with the plant extract at the concentration of 2.0 gm/kg feed showed the highest percentage (97.43 ± 0.13) of males, which was significantly higher ($P < 0.05$) compared to the other two concentration categories and control group (43.67 ± 2.75). Interestingly, treatment with the higher concentrations of 1.0 and 3.0 gm/kg feed produced only (94.33 ± 0.23) and (95.27 ± 0.62) males, respectively. These results agree with that recorded by **Kavitha and Subramanian (2011)**. Such reduction in percentage of males with increase in dietary concentration of the plant powder and plant extract was also reported during treatment of mixed-sex tilapia (**Ghosal et al. 2015, 2016**). Reduced masculinization and paradoxical feminization have been observed in fish treated with high concentration of synthetic steroids as well (**Devlin and Nagahama, 2002**). These results agree with that recorded by **Neychev and Mitev (2005)** and **Mukherjee et al., 2018**. In another study, however, dietary inclusion of commercially available *Tribulus terrestris* extract at a concentration of 2.5 g/kg basal diet has resulted in 84% male population in *O. niloticus* (**Omitoyin et al. 2013**). Dietary treatment with ethanol extract of *Tribulus Terrestris* seeds at the concentration of 1.5 g/kg feed has resulted in 89% males in Nile tilapia (**Ghosal et al. 2015**).

Increase in percentage of males has been observed by increasing the plant extract concentration to 2.0 g/kg feed in the present study. Steroidal saponin protodioscin might be considered to be the androgenic bioactive phytoconstituent in *Tribulus Terrestris*

ethanol extract (**Gauthaman and Ganesan, 2008**). But the mechanism of masculinization by the plant extract is yet to be deduced (**Gauthaman, et al., 2002 and Dada, 2015**).

The present results suggests that supplemented *T. terrestris* extract in feeds for Nile tilapia improves growth performance and hatching rate; we conclude that *T. Terrestris* extract in lower dosage has the potential to be used as a supplement in fish diets. The use of medicinal plants in fish will be an efficient tool to achieve sustainable, economical, and safe monosex fish production. Dietary treatment with *Tribulus Terrestris* aqueous extract at a concentration of 2.0 g/kg feed for 30 days may be implemented for production of almost male tilapia population.

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