PROMOTING AQUACULTURE THROUGH PHYTOCHEMICALS: THE ROLE OF PUNCTURE VINE (*Tribulus terrestris* L.) IN AQUACULTURE (A REVIEW)

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ABSTRACT
The increasing demand for fish and fish products necessitates advancements in aquaculture, particularly in Sub-Saharan Africa, where the industry is experiencing rapid growth. Nigeria, a leading aquaculture producer in Africa, is seeing a shift in consumer preference from catfish to tilapia, despite the sector's limited exploitation of technology. To meet global fish demand by 2050, aquaculture production must double, emphasizing the need for sustainable and efficient methods to enhance fish growth and production. This study explores the potential of using Tribulus terrestris, a medicinal plant known for its growth-promoting and sex-reversal properties, in aquaculture. Tribulus terrestris contains various bioactive compounds, including alkaloids, flavonoids, and sapogenins, which exhibit anti-inflammatory, immunomodulatory, and antioxidant activities. Previous research indicates that T. terrestris extracts can significantly improve growth and body weight in various fish species, such as Clarias gariepinus, Oreochromis niloticus, and Poecilia reticulata. Additionally, T. terrestris has demonstrated potential in inducing sex reversal, a critical aspect of optimizing aquaculture production. This paper reviews the biological, therapeutic, and toxicological characteristics of T. terrestris, highlighting its application in aquaculture. It further reviewed the optimal dosages and methods of administering T. terrestris extracts to achieve enhanced growth performance, improved health parameters, and effective sex reversal in fish. The findings suggest that integrating T. terrestris in aquaculture practices could significantly boost production, ensuring food security and economic benefits.

Keywords: Bioactive compounds, fish farming, caltrop, sustainable farming

INTRODUCTION

Increased demand for fish and fish products will mostly be met by growth in supply from aquaculture (FAO, 2016). In freshwater aquaculture, tilapia has become the most cultivated group of fishes around the world, with the second highest production, after carp (FAO, 2016; Waite *et al.*, 2014). It is estimated that, for fish availability to meet projected demand worldwide, aquaculture output must double by 2050 through improving aquaculture that can result to significant food security and developmental benefits (Waite *et al.*, 2014). Sub-Saharan Africa is said to have the fastest growing aquaculture industry with reporting that Sub-Saharan Africa witnessed a 14.5% growth in aquaculture, going from 346,400 tonnes in 2019 to 396,700 tonnes in 2020 with almost 12% of all farmed aquatic species in Africa in 2020 were produced in Nigeria FAO (2022).

Despite Nigeria being second leading aquaculture producer in Africa, having a strong aquaculture growth, contributing 0.4% of the world's total aquaculture production, aquaculture is the least exploited fishery sub-sector in Nigeria (FAO, 2018). Although, Nigeria is one of the leading countries in catfish

production in Africa, Tilapia is quickly becoming the most preferred fish by consumers as evidenced in the increase of Tilapia production from 68,621 tonnes in 2010 to 95,319 tonnes in 2015 compared to the decline in catfish production from 138,300 tonnes to 112,300 tonnes within the same period (NBS, 2017).

Several researches abound on the use of phytochemicals as growth promoters in animals including fish. This is against the backdrop of the constant need to improve productivity especially in aquaculture. Though plant chemicals have been used as food flavours, preservatives and medicines, the fundamental mechanism of the use of these as growth promoters or enhancers have not been clearly elucidated yet (Valenzuela-Grijalva *et al.*, 2017). Many have asserted that, the use of plant extracts as alternatives to synthetic anabolic hormones have proven to be effective, safer and more efficient, purporting that, the potency of the antioxidants as well as immuno-stimulating actions of these plant chemicals can enhance fish growth, however, the presence of anti-nutritional factors in these plants have limited the use of these sources in fish feed (Chakraborty, 2017; Chakraborty *et al.*, 2012). Therefore, there is the need to review the applications of plant extracts such as *Tribulus terrestris* extract in aquaculture.

Tribulus terrestris L. belonging to the family Zygophylaceae, is commonly known as puncture vine and in Nigeria as, tsaiji in Fulfulde; hana taakama and tsaida in hausa and da ogun daguro in Yoruba (Chhatre *et al.*, 2014 and Muanya, 2015). It is made up of different constituents such as; alkaloids, flavonoids and sapogenins, which have several properties like anti-inflammatory, immunomodulatory, anti-tumor activities and other far-ranging biochemical reactions (Rakesh *et al.*, 2009; Kavitha *et al.*, 2011; Gültepe *et al.*, 2014).

Description, Phyto-constituents, Uses and Toxicity of *Tribulus terrestris*

Biological description of *T. terrestris*

Tribulus terrestris is a four-carbon carbon(iv)oxide photosynthetic (C_4) herbaceous, mat forming, annual plant of the family Zygophyllaceae family (Nikolova and Vassilev, 2011). This is an annual herb with prostrate creeping branches with a semi-perennial underground stem and root system that is widely distributed in both tropical and warm temperate countries (Africa, China, India, Japan, Korea, Turkey, southern Europe and western Asia) and grows as a summer annual plant in colder climates (Akram *et al.,* 2011).

Tribulus terrestris has an even-pinnate compound, opposite leaves that is approximately 1 – 5 cm long, with 3 – 7 pairs of elliptic or oblong leaflet per leaf that are covered with uniseriate epidermis with polygonal epidermal cells and small anomocytic stomata (Nikolova and Vassilev, 2011). *Tribulus terrestris* has a seed dormancy period of several months with seedlings emerging at the start of the rains with increased moisture in the soil. The seedlings develop a deep root system within a short period, producing flowers and fruits in three and six weeks respectively (Moradikor *et al.*, 2013). *Tribulus terrestris* has slender, branched, woody and fibrous deep taproot with potential to develop nitrogen-fixing nodules (Nikolova and Vassilev, 2011), this deep taproot provides the mechanism for acquiring more water. Nikolova and Vassilev (2011) concluded that the anatomical structure of the vegetative parts of *T. terrestris* showed adaptations typical for both drought-evading and drought-enduring species.

Once *T. terrestris* plant begins to bloom, it continues throughout. The flowers are cross pollinated by insects as well as being self-pollinated, typically bearing numerous angular schizocarp capsular fruits divided into five burrs with each fruit containing fiving nutlets which in

turn, bears two to three seeds (Moradikor *et al.*, 2013). *Tribulus terrestris* fruits attach easily to animals and humans via two distinct sharp divergent spines and many other tiny spines (Rodriguez-Fragoso *et al.*, 2008).

Tribulus terrestris seeds are flat, triangle or oval in shape with a long-pointed top and a flat base with well-developed round embryo (Semerdjieva and Zheljazkov, 2019) and many new matured seeds are dormant and uses about a year to fully ripen. *Tribulus terrestris* seeds germinate at a temperature between 27^{0}C to 35^{0}C, low temperature and light intensities as well as wet soil can inhibit these seeds germination. *Tribulus terrestris* was observed to germinate and emerge following a rain shower having more than 10 mm of precipitation (Ernst and Tolsma, 1988), these authors also reported that, 35% seeds germination occurred after many heavy rains.

Phytochemical constituents of *T. terrestris*

A number of medicinal plants have been used by man for so many reasons, since the beginning of human civilization (Petrovska, 2012). Thus, attempts to use the natural materials such as medicinal plants as feed additives to achieve sex-reversal, enhance efficiency of feed utilization and animal productive performance has been widely accepted as reported by Dada (2015) that, use of plant-based additives in aquaculture is one of the methods used to improve weight gain and feed efficiency in cultured fish. One of such plants to be considered is *Tribulus terrestris* and like all medicinal plants, *Tribulus terrestris* contains various phytochemical constituents and have been reported extensively by several researchers (Table 1). These reports may vary depending on geographical region, growth condition, ecotype as well as methods of extraction (Sansebastiano *et al.*, 2013; Farooq *et al.*, 2012).

The chemical constituents of fruits of *T. terrestris* have been studied more than other parts of the plants, this is followed by other aerial parts and the vegetative parts, studied least (Semerdjieva and Zheljazkov, 2019).

Table 1. Phytochemicals in T. terrestris

S/No.	Phytochemical	Part of *T. terrestris*	Extraction Solvent	Reference
1	alkaloids, flavonoids, glycosides, saponins, and tannins	Leaves	Methanol	Usman *et al.*, (2007)
2	saponins, flavonoids, alkaloids	Whole plant	Water	Gauthaman and Ganesan, (2008)
3	alkaloids, carbohydrates, flavonoids, saponins and tannins	Leaves and fruits	Water and methanol	Sharma *et al.* (2013)
4	alkaloids, proteins, steroidal saponins and sugars	Fruits	Water and methanol	Mathur and Sundaramoorthy (2013)
5	alkaloid, tannins, saponins, carbohydrates, flavonoid, terpenoids, steroids, anthraquinone, glycosides, and sterols	Flowers	Water, ethanol and benzene	(Sujatha and Prakash, 2013)
6	flavonoids, saponins, tannins and terpenoids	Whole plant	Ethanol	Gincy *et al.* (2014)
7	Alkaloids, glycosides, saponins, tannins, flavonoids, phenolic compounds and carbohydrates	Fruits	Water and Ethanol	Pandey (2014)

8	Alkaloids, flavonoids, glycosides, lignin, amides and saponins	Whole plant	Water and Methanol	Shahid *et al.* (2015)
9	Saponins, flavonoids, alkaloids, glycosides and phytosteroids	Whole plant	water, methanol, and chloroform	Abudayyak *et al.* (2015)
10	Amino acids, glycosides, carbohydrates, phenols, terpenoids tannins, proteins, saponins,	Leaves and fruits	Water, acetone and methanol	Vasait, (2017)
11	Alkaloids, flavonoids, saponins, steroids, glycosides and phenolic compounds	Fruits, leaves and roots	chloroform, ethanol, water and petroleum ether	Dwivedi and Sengar, (2018)
12	Sterols, terpenes, flavonoids, saponins, alkaloids and nitrogenous compounds	Dried aerial parts	Ether, petroleum ether, methanol and aqueous methanol (50%)	Ammar *et al.* (2018)
13	Terpenoids, saponins, tannins, alkaloid, steroids, glycosides and phenols	Whole plant	Chloroform, Petroleum ether, Acetone, Methanol, Ethanol, Ethyl acetate and water	Maheswari and Rajendran (2018)
14	Alkaloids, cinnamic acid amides and lignan amides	Whole plant	Water	Semerdjieva and Zheljazkov (2019)
15	Steroid, terpenoids, flavonoids, saponins, alkaloids, anthraquinones	Whole plant	Water, Ethanol and Ethyl acetate	Sadiq (2021)

Therapeutic applications of *T. terrestris*

Tribulus terrestris is a wide-ranging medicinal plant utilized by athletes majorly as a result of the economic claims (Pokrywka *et al.*, 2014). The whole of the aerial parts of *T. terrestris*; leaves, fruits, flowers and stem have been reported as to have medicinal properties and have been used for such purposes and these have no taste or smell (Semerdjieva and Zheljazkov, 2019). Initial researches revealing the beneficial effects of *T. terrestris* were carried out by a drug producing company in Bulgaria. Early results showed that supplementary use of *T. terrestris* raised plasma testosterone, leading to increase in lean mass and strength implying a vital effect resulting from the naturally occurring saponins in *T. terrestris*, which are akin to conventional mechanisms and androgenic routes like the release of Luteinizing hormones and testosterone (Pokrywka *et al.*, 2014).

Toxicity of *Tribulus terrestris*

Medicinal plants have often been claimed to safe, however, many studies have stated that various plants used as food, spices or in traditional medicine have endocrine disrupting, carcinogenic or mutagenic properties (Abudayyak *et al.*, 2015). Although, *T. terrestris* has a wide range of uses across many cultures and countries, some studies have dwelt on the toxic potential of this plant.

Talasaz *et al.* (2010) reported a case of a young man hospitalized with symptoms of neurological disorders renal lesions and hepatitis after consuming large doses of *T. terrestris* during a period of two days who then recovered from these symptoms after ceasing *T. terrestris* consumption. According to Ryan *et al.* (2015), a man, 30 years old, started experiencing nausea, weakness and poor appetite after commencing *T. terrestris* extract tablets as part

of his body building regime, this continued over a period of six weeks before seeking medical attention, he soon recovered after stopping *T. terrestris* consumption. Abudayyak *et al.* (2015) determined the safety of *T. terrestris*, on mutagenicity, DNA damage, and disruption of endocrine which resulted in methanol extracts of *T. terrestris* reducing cell viability while the water and chloroform extracts showed no cytotoxic effects. This report was similar to that of Angelova *et al.* (2013) who found out that Bulgarian *T. terrestris* extract had a significant dosage dependent inhibitory effect on the viability of breast cancer cells.

Other researches have shown methanol *T. terrestris* fruit extracts to have inhibitory effects (LC_{50}) on Dalton's Lymphoma Ascites and Ehrlich's Ascites Carcinoma in mice at 380 µg/ml and 420µg/ml respectively (Divya *et al.*, 2014). In humans, the growth of human prostate cancer, cervical cancer line and breast cancer were inhibited by different extracts of *T. terrestris* parts in different concentrations (Wei *et al.*, 2014; Dhanalakshmi *et al.*, 2016 and Patel *et al.*, 2016). In vitro and in silico studies of Basaiyye *et al.* (2018) showed that alkaloids from *T. terrestris* possessed cytotoxic and pharmacological properties which may induce extrinsic and intrinsic apoptosis pathways in leukemic cell line.
In treatments with *T. terrestris*, Assunção *et al.* (2017) suggest that small doses should be administered to allow for elimination and metabolic breakdown of saponins, since huge doses may lead to plasmic collection and induction of membrane rupture as well as death of erythrocytes.

Uses of *Tribulus terrestris* in Fish and other animals

***Tribulus terrestris* extract as a growth promoter in fish**

Tribulus terrestris have been acclaimed by several researchers to support growth in other animals and fish (Table 2), this was further substantiated by the reports of Abadjieva and Kistanova (2016) that *T. terrestris* altered the growth differentiation factor 9 (GDF 9) in New Zealand female rabbits which were inherited by the F_1 generations, they concluded that, GDF 9 were sensitive to *T. terrestris* which resulted in an increase of GDF 9 at mRNA and protein levels of oocytes and cumulus cells. Growth of fish is one of the factors that can lead to improved productivity in aquaculture particularly in Nigeria and other developing countries (Sadiq, 2021). The growth promoting effects of *T. terrestris* extract were recorded during immersion experiments on convict cichlid, *Cichlisoma nigrofasciatum* and guppy, *Poecilia reticulata* (Çek *et al.* 2007a; 2007b). These authors reported that, *T. terrestris* extract treated fish exhibited successful growth acceleration and significantly higher growth rates compared with the control group, these results corresponded with the findings of Turan and Cek (2007) who observed the highest body weight gain in *Clarias gariepinus* treated with 9 g *T. terrestris* extracts for 30 days.

Kavitha and Subramanian (2011) measured the growth rate of 0-day old *Poecilia latipinna* fry immersed in water containing 0 ppm/L, 10 ppm/L, 15 ppm/L, 20 ppm/L, 25 ppm/L and 30 ppm/L of *T. terrestris* ethanol extract two months. They reported accelerated growth rate in terms of total length and body weight in all the treated fish compared with the control group with those treated with 30 ppm/L producing the highest growth rates. Yilmaz *et al* (2013) treated all-female population of *Oncorhynchus mykiss* with 50 mg/kg and 100 mg/kg *T. terrestris* along with treatments of testosterone and methlytestosterone and achieved 117.09g of weight gain in the 100mg/kg *T. terrestris* treated fish which was significantly higher than those of other treatments. Gultepe *et al.*, (2014) also reported an enhanced growth performance in *Oreochromis niloticus* fed different levels between 200mg/kg to 600 mg/kg of *T. terrestris* extracts. Similarly, Omar *et al.* (2014) fed *O. niloticus* diets with 0.6 g/kg and 1.2 g/kg *T. terrestris* extracts to ascertain the impact of this dietary addition of *T. terrestris* extracts on the growth performance of the fish after 28 and 84 days. At the end of 28 days, fish fed with 1.2 g/kg *T. terrestris* had the highest final body weight and specific growth rate while at 84 days, fish fed MT diets had the highest growth rate followed

by those fed diets with 1.2 g/kg with no significant differences between them. They concluded that, growth of *O. niloticus* can be enhanced using *T. terrestris* extracts.

Yeganeh *et al.*, (2017) also reported a significantly higher weight gain as well as specific growth rates in male *C. nigrofaciatum* fed diets supplemented at 1g/kg (123.83g and 1.76%) and 2g/kg (121.13g and 1.79%) for 45 days compared to fish in the control group having 80.1g weight gain and 1.3% specific growth rate. *Tribulus terrestris* extract when mixed with *U. dioica* extract at a concentration of 10mg/L *T. terrestris* and 200mg/L *U. dioica* was reported to improve growth indices of *C. nigrofaciatum*, this was against the backdrop that growth of *C. nigrofaciatum* declined when treated with only *U. dioica* extracts (Babahajiani *et al.*, 2018). Sadiq (2021) recorded the highest growth in *O. niloticus* fed diets containing 2.5g/kg *T. terrestris* aqueous and ethanol extracts, however, the author concluded that this growth was not significantly different from fish fed 2.0g/kg *T. terrestris* aqueous extracts.

Table 2: *Tribulus terrestris* extract as growth enhancement agent in fish

S/No.	Part used	Effective concentration	Reference
1	*Cichlisoma nigrofasciatum*	0.30 g/L	Çek *et al.* (2007a)
2	*Poecilia reticulate*	100 mg/L	Çek *et al.* (2007b)
3	*Clarias gariepinus*	9 g/kg	Turan and Cek (2007)
4	*Poecilia latipinna*	30 ppm/L	Kavitha and Subramanian (2011)
5	*Oncorhynchus mykiss*	100 mg/kg	Yilmaz *et al.* (2013)
6	*Oreochromis niloticus*	2.5 g/kg	Omitoyin et al. (2013)
7	*O. niloticus*	600 mg/kg	Gultepe *et al.* (2014)
8	*O. niloticus*	1.2 g/kg	Omar *et al.* (2014)
9	*C. nigrofaciatum*	2 g/kg	Yeganeh *et al.* (2017)
10	*C. nigrofaciatum*	10 mg/L	Babahajiani *et al.* (2018)
11	*O. niloticus*	2.0 g/kg	Sadiq (2021)

Evidence of *T. terrestris* improving sex hormone in animals and use for sex reversal in fish

Tribulus terrestris has long been a constituent in traditional medicine, where in it is used as an aphrodisiac, also used diuretic and nervine in tonic, where as in Unani medicine to inhibit the formation of kidney stone (Aggarwal *et al.,* 2010). *Tribulus terrestris* reportedly increased sperm count and sperm motility after it was consumed for a month by studies of Akram *et al.* (2011) which led to the conclusion that *T. terrestris* is a reliable supplement for males and females to raise their libido. Some clinical studies showed *T. terrestris* improved reproductive function, including increased concentration of hormones such as estradiol, with testosterone being very slightly influenced, thereby improving reproductive function, libido and ovulation (Shaheen *et al.,* 2012).

The use of medicinal herbs in place of chemicals and drugs have been lauded in many researches as being more beneficial and suitable alternatives (Gabriel *et al.*, 2015; Ghosal and Chakraborty, 2014a; 2014b; Hu *et al.*, 2014; Felicitta *et al.*, 2013; Bai *et al.*, 2012). Some

phytochemicals in plants known as phytoestrogen have been reported by Das *et al.* (2012) to imitate or serve as sex hormones where they function to obstruct the biological production of estrogen in gonadal germ cells, this led the authors to conclude that, these phytochemicals can potentially be used to cause sex reversal or delay maturity in fish. Herbal supplements are efficacious and more ecofriendly in health management that improves non-susceptibility to disease-causing microorganisms (Harikrishnan *et al.*, 2010). Medicinal plants can be administered to fish and shellfish by injection (intra-muscular and intra-peritoneal), oral administration and through immersion or baths (Putra *et al.*, 2013; Ji *et al.*, 2012; Wu *et al.*, 2010).

One of the most popular medicinal assertions of *T. terrestris* use its potential to improve libido and erectile dysfunction, however, Santos *et al.* (2014) reported studies with adult male and female individuals where there were no significant results in the male individual but a considerable libido improvement in the female individuals. An indication of *T. terrestris* stimulating a rise in cyclic monophosphate adenosine and cyclic monophosphate guanosine levels in *corpus cavernosum* of *in vivo* models by Do *et al.* (2013), suggests that *T. terrestris* can stimulate production of nitric oxide in the tissue. Roaiah *et al.* (2015) assessed the effect *T. terrestris* on serum testosterone, luteinizing hormone and erectile function of male subjects with partial androgen inadequacy, the authors observed a significant difference in the testosterone levels and erectile function of the subjects after treating them with 250mg *T. terrestris* extract for 90 days.

The hormonal effects of rabbits and rats were treated with 2.5 $mgkg^{-1}$, 5 $mgkg^{-1}$ and 10 $mgkg^{-1}$ of *T. terrestris* extract orally for 8 weeks were investigated by Gauthaman and Adaikan (2008), the results indicated that testosterone and dihydrotestosterone levels were increased in the rabbits. Likewise, in castrated rats, testosterone levels increased by 25% after treatment with *T. terrestris* extracts at 5 $mgkg^{-1}$ daily for 8 weeks. There was limited study on whether *T. terrestris* extract affects growth rate and body muscle in broiler chicks since these body parts are of significant economic importance on broiler meat production. This led Şahin and Duru (2010) to study the effects of *T. terrestris* supplementation on performance and digestive system of broiler chicks. El-Shaibany *et al.* (2015) assessed antihyperglycaemic properties of *Tribulus terrestris* extract in glucose loaded rabbits and found that, a single dose of methanol extract of *T. terrestris* at 250 mg/kg significantly lowered fasting blood glucose by over 50% in hyperglycaemic rabbits, concluding that, this antihyperglycaemic property might be due presence of saponins in *T. terrestris*. Saiyed *et al.* (2016) treated diabetic rats with ovarian polycystic syndrome with a combination of hydroacoholic extracts of *Withania somnifera* and *T. terrestris* at 198mg/kg for 21 days and reported that the combination of *W. somnifera* and *T. terrestris* extracts were able to return to normalcy the hormonal levels of tested animals, concluding that the hydro alcoholic extracts of these plants exhibited significant recovery of estradiole, follicle stimulating hormones, luteinizing hormone and testosterone levels. More recently, Servati *et al.* (2016) noted that Duru (2005), gave broiler chicks *T. terrestris* extract orally in commercial diet, stating that, there was no effect on growth performance and body parts. Likewise, Amirshekari *et al.* (2015) examined the effects on the blood parameters and productive performance of laying hens fed aqueous extract and dried aerial parts of *T. terrestris*. They added 5 mg and 10 mg to both drinking water and feed of the hens. They reported that, there was an improved feed conversion ratio of the hens leading to better performance with increased reproductive performance as well. Finally, the administration of *T. terrestris* to both humans and animals improves libido and spermatogenesis (Kotta *et al.*, 2013). In men, it was utilized in the

treatment of erectile dysfunction and in raising the levels of testosterone while improving athletic performance (Singh *et al.*, 2012 and Porkrykwa *et al.*, 2014). Several other researches on the influences of *T. terrestris* extracts on sex hormones for different animals have been reported and recorded over the years (Semerdjieva and Zheljazkov, 2019).

The concept of the use of phytochemicals to induce sex reversal in fish is against the back drop that studies have revealed that phytochemicals can impede biological production and estrogen action by functioning as aromatase inhibitors and nuclear estrogen receptor antagonists in gonad germ cells (Mukherjee *et al.*, 2018). Many researchers have reported that *Tribulus terrestris*, a medicinal plant tend to increase testosterone levels which improves androgen metabolism (Babahajiani *et al.*, 2018; Yeganeh *et al.*, 2017; Farooq *et al.*, 2012). With increased demand for organic fish and fish products world over, the application of plant extracts in every aspect of fish farming may not only boost production, but it could improve the quality and safety of fish and fishery products, which would in turn lead to higher confidence in accepting these products worldwide (Gabriel *et al.*, 2015). *Tribulus terrestris* is ingested by people, allegedly for muscle building and has demonstrated hepato-protective and antioxidant activities in *O. mossambicus* (Kavitha *et al.*, 2011).

Sex differentiation process in fish and hormone production can be impacted by phytochemicals and the temperature of water which has led many authors to surmise that, these factors can provide other means of single sex fish populations in fish farming (Fuentes-Silva *et al.*, 2013 and Baroiller *et al.*, 2009). Plant extracts have been found to show dose-dependent variability in many functions (Mahomoodally, 2013). Kavitha and Subramanian (2011) experimented by immersing 0-day old *Poecilia latipinna* fry in water containing varying concentrations of *T. terrestris* extracts ranging from 0 ppm/L to 50 ppm/L for 60 days. The authors reported a 97% masculinization in fry immersed in 50 ppm/L stating that, the sex ratio in this group of fish (97:3; male : female) was significantly higher than those of fry treated in other concentrations. Yilmaz *et al.* (2013) fed an all-female population of *Oncorhynchus mykiss* with feed supplemented with 50 mg and 100 mg *T. terrestris* extract as well as 3mg and 6mg of both testosterone and methyltestosterne per kilogram for 90 days. They recorded between 55% to 63% males in the population and concluded that, *T. terrestris* extract was effective in inducing masculinization compared to testosterone. Omitoyin *et al.* (2013) achieved 83.7% masculinization of *O. niloticus* with commercial *T. terrestris* extract at 2.0g/kg.

Similarly, Ghosal and Chakraborty (2014) recorded 81.4% males in treating *O. niloticus* with *T. terrestris* aqueous extracts at 1.5g/kg while Omar *et al.* (2014) achieved 64.48% and 57.76% males percentage after feeding *O. niloticus* diets with 0.6 g/kg and 1.2 g/kg *T. terrestris* extracts respectively. Ghosal *et al.* (2015) reported a dose dependent increase in masculinization when treated with increasing concentration of ethanol extract of *T. terrestris* seeds on Nile tilapia and the highest percentage (89%) of males has been achieved at the concentration of 1.5 g/kg feed. Yeganeh *et al.* (2017) further tested the effects of *T. terrestris* on the reproductive performance of male convict cichlid *Cichlasoma nigrofasciatum* which resulted in highest fertilization and hatchability in fish fed with 1 g/kg of *T. terrestris* compared to the control and those fed 2 g/kg. Janalizadeh *et al.* (2019 and 2018) also assessed the effects of *T. terrestris* by immersion and feeding on *Betta splendens*. The authors fed *B. splendens* and *T. terrestris* enriched artemia and dipped another group water containing *T. terrestris* extracts ranging from 0.00g/L to 0.05g/L concentrations and reported that, *B. speldens* had higher male percentage and survival rates in those fed with artemia enriched in 0.05g/L *T. terrestris* compared to other

groups, stating that, feeding *T. terrestris* enriched artemia produced better results than immersion. Considering all these, the aim of the present experiment was to determine whether application of further higher doses of *T. terrestris* extracts using different solvents would yield higher percentage of male tilapia. Sadiq (2021) achieved 90.48% males in O. niloticus treated with aqueous extracts of *T. terrestris* and 87.62% males in those treated with ethanol extract of *T. terrestris* respectively. The author concluded that, having observed highest males percentage in fish treated with 2.0g/kg *T. terrestris* aqueous extract, it would be counterproductive to treat fish with a higher dose.

Conclusion

In conclusion, the increasing demand for fish and fish products is primarily being met by the growth of aquaculture, with tilapia emerging as a significant species in freshwater aquaculture globally. Sub-Saharan Africa, and particularly Nigeria, is witnessing rapid growth in this sector. Despite the promising trends, the potential of aquaculture remains underexploited, particularly in Nigeria. One promising area for enhancing aquaculture productivity is the use of phytochemicals, such as Tribulus terrestris extracts, which have shown potential as growth promoters and sex-reversal agents in fish. While the use of T. terrestris in traditional medicine is well-documented, its application in aquaculture offers a natural alternative to synthetic hormones, potentially improving fish growth, health, and production efficiency. The effectiveness of T. terrestris in promoting growth and inducing sex reversal in various fish species underscores its potential for broader application in the industry. Future research should continue to explore optimal dosages and methods of administration to maximize the benefits of T. terrestris in aquaculture.

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