SEX REVERSAL OF NILE TILAPIA *Oreochromis niloticus* (LINNEAUS, 1758) USING DIFFERENTLY PROCESSED *Tribulus terrestris* L. EXTRACTS

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A Thesis in the Department of Aquaculture and Fisheries Management,
Submitted to the Faculty of Renewable Natural Resources
in partial fulfillment for the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

AUGUST 2021

CERTIFICATION

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DEDICATION

This project is dedicated to God who has kept me through all my years of studies and to my daughter, Zoe Ahuoiza Omeiza.

ACKNOWLEDGEMENTS

My unalloyed gratitude goes to God for all the grace and providence He showered on me during my studies and for the successful completion of this thesis.

My deepest appreciation indubitably goes to my mentor and supervisor, Prof. B. O. Omitoyin for his patience, interest, help, guidance and encouragement not just during this project alone, but throughout my studies at the University of Ibadan. Sir, your advice all those years ago still resound in my mind and has always guided me in many decisions I have made, thank you very much sir. I also send my unreserved gratitude to Prof E. K. Ajani who has been a strong pillar to the successful completion of this work, may God reward you abundantly. Many thanks to Profs. A. O. Akinwole, and Adetola Jenyo-Oni, Drs. Siyanbola A. Omitoyin, Flora E. Olaifa, O. K. Kareem and F. E. Osho and indeed all lecturers and staff of the Department for all their individual and collective contributions towards the success of this work. God bless you all. I must recognize the assistance and guidance of Prof. Ofelia G. Omitogun during the molecular analysis of this study, Mama, you and your entire laboratory team were immensely instrumental to the success of this study, I am grateful. I also thank Dr. H. Osaiyuwu for his assistance.

My profound gratitude goes to my parents Mr. and Mrs. I.A. Sadiq, my dearest brother Hans and the rest of my family for all the love, care and support financially, morally and spiritually. I would not have come this far without you all. Special thanks to my husband, Mr. Abdullahi Omeiza, thanks for being my pillar and support.

Others who have stood by me in the times of my ups and downs during the course of my studies at the University of Ibadan and supported me in one way or the other, His Grace, Archbishop Gabriel Agbegurin, Fathers Owoeye, Kwaghgba, and Ojo, Prof. B. O. Emunemu, Nkechi, Davina and Pamela, Christyand the entire Eragbe family, Drs. Magomya, and Obani, Profs. Ogunremi and Ayuba, Celestina, Judith, Francis, Felix, Iwezulu, Ubom, Maryann, Eugene and Omo, Linda, Gloria, Niyi, Grace, Yewande, Kemi, Omime and my colleagues in the Department of Fisheries and Aquaculture, Federal University Wukari, for their friendship, support and prayers, I am eternally grateful. My classmates, my other colleagues and NFCS UI alumni family, for lack of

space, I say thank you for all your support, I appreciate you all and wish you the very best. God bless you all.

ABSTRACT

All male monosex culture in *Oreochromis niloticus* (ON) has been identified as a means of eliminating problem of stunted somatic growth caused by their prolific breeding habit. Production of all male ON populations through the use of synthetic steroid hormones has generated environmental and public health concerns, hence the need for natural alternatives. Phytochemicals from plants such as *Tribulus terrestris* (TT) has been reported as potential means of inducing sex reversal in fish. However, the efficacy of TT extracts for production of all male ON needs to be documented. Therefore, the effectiveness of TT extracts for sex reversal and growth enhancement of ON larvae was investigated.

Extracts of TT obtained using ethanol (TTE), ethyl acetate (TTEA), aqueous (TTA_{100})at100^0C and (TTA_{27}) at 27^0C (room temperature) were assessed quantitatively for phytochemicals. Two extracts with highest saponin and steroid contents were used for the nutritional study. Each extract was added to isonitrogenous diets (Crude Protein 45%) at 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g/kg while diet supplemented with 0.5mg/kg of 17-α-methyltestosterone (MT) served as control diet. *Oreochromis niloticus* larvae (n=3150; 0.02±0.001g) were randomly allotted to treatments in triplicates in a 2x7 factorial design. Fish were fed to satiation for 126 days. Percentage males (PM) in randomly selected fish (n=210) were determined using sex-linked markers. Growth performance indices such as Mean Weight Gain (MWG) and Specific Growth Rate (SGR) were determined. Blood (5mL) was sampled to evaluate Haematocrit, Aspartate aminotransferase (AST) and Serum Cholesterol (SC) using standard procedures. Data were analysed using descriptive statistics, ANOVA and Polynomial regression at $\alpha_{0.05}$.

Flavonoid, saponins, anthraquinones, terpenoids and steroids were present in all TT extracts. Saponin was significantly highest in TTA_{27} (83±0.0mg/g) while steroid was highest in TTE (19±1.0mg/g). Least saponin (1±0.0mg/g) and steroid (3±0.00mg/g) were obtained in TTEA. Percentage males were 87.62±2.02% and 90.48±2.0% for TTE and TTA_{27} respectively. Least (53.33±11.6%) and highest (100.00±0.0%) PM were obtained in 0.0g/kg and 1.5g/kg TTA_{27},respectively. Least (3.56±0.4g; 4.110±0.1%/d) and highest (3.73±0.4g; 4.156±0.1%/d) MWG and SGR were recorded in TTE and TTA_{27}, respectively. The MWG varied significantly from 2.26±0.2g (0.0g/kg) to 4.80±0.2g (2.5g/kg TTA_{27}), while SGR were 3.79±0.1%/d and 4.39±0.03%/d in 0.0g/kg and 2.5g/kg TTA_{27}, respectively. Optimum inclusion level of TTA_{27} relative to MWG was 2.05g/kg (R^2=0.95). Haematocrit ranged from 25.36±0.1% (TTE) to 25.37±0.1% (TTA_{27}) while AST was 64.14±0.1µ/L (TTE) to 64.30±0.1µ/L (TTA_{27}). Highest (25.66±0.01%) and least (25.12±0.02%) haematocrit were recorded in 0.5g/kg TTA_{27} and 1.0g/kg TTE, respectively. The AST was highest (64.94±0.0µ/L) in 0.0g/kg and least (63.57±0.6µ/L) in 0.5g/kg TTE. The SC ranged from 141.34±0.1g/dL (TTA_{27}) to 142.08±0.1g/dL (TTE). Significantly highest SC (162.60±0.3g/dL) was recorded in MT while 2.5g/kg TTA_{27} gave the least (134.44±0.16 g/dL).

Tribulus terrestris water extract at 1.5g/kg could optimally reverse sex of *Oreochromis niloticus* larvae. Diets containing *Tribulus terrestris* water extracts at inclusion level of 2.05g/kg enhanced growth and health of *Oreochromis niloticus* larvae.

Key words: Puncture vine, *Oreochromis niloticus*, Monosex culture, Sex-linked markers.

Word count: 473

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CHAPTER ONE
INTRODUCTION

1.5 Background of the study

Tilapia has become the most cultivated group of fishes around the world, with the second highest production, after carp (FAO, 2016a; Waite *et al.*, 2014). It is critical to have a good understanding of the basic features of Nile tilapia's biological cycle, particularly reproductive characteristics, for commercial production. The success of Nile tilapia culture results from the excellent adaptation of this group of fishes to industrial culture conditions but their reproductive precocity seems to require control measures to prevent the diversion of energy from muscle production to reproduction. It appears that some of the most frequently used reproductive control measures involve monosex cultures through sex reversal, interspecific hybridization, and manual sex selection (Ramirez *et al.*, 2017).

Fish like other aquatic organisms constitute a matchless contribution to food as well as nutrition security in many African countries where many people are poor and undernourished (Belton and Thilsted, 2014). The exceptional blend of high-quality protein and other essential nutrients makes fish a unique valuable food. It is a good source of animal protein, it is estimated that about 140/150g of fish provides nearly 60% of the daily requirements of man. Fish also provides some other essential nutrients such as fatty acids, vitamins, iodine and selenium, which are non-existent or insufficient in other protein sources like cereals and meat (Beveridge *et al.*, 2013; Kawarazuka and Béné, 2011). With an approximate serving size of 150 g, this leads to a worldwide recommended yearly consumption of about 12 kg fish per capita (WWF, 2016). This value has however risen above 20kg/year (FAO, 2016a). Fish and other products from fish therefore serve as a very important avenue of nutritional protein and other necessary micro nutrients for a good, complete nutrition and health (FAO, 2012).

Currently, Nigeria’s total fish production is 1,027,058 tonnes (NBS, 2017) while the demand for fish is about at 3.32 million tonnes (FCWC, 2016) leaving a deficit of over 2

million tonnes. According to FAO (2008; 2010), capture fisheries production has been on the decline from the year 2000 to 2008 (95.6 - 89.7 million tonnes) while aquaculture production has been on the increase from 35.5 million tonnes in 2000 to 52.5 million tonnes in 2008. This is evident in Nigeria, as fish production (artisanal and industrial) dropped from 809,780 tonnes in 2014 to 710,331 tonnes in 2015 while an increase from 313,231 tonnes in2014 to 316,727tonnes in 2015 resulted from aquaculture production (NBS, 2017). A 32.1% growth projection in Nigeria's total fish production in 2025 of which 89.3% will be from aquaculture compared to the average of 2013 to 2015 while per capita fish consumption will increase by 5.9% has been estimated by FAO (2016a). Allen *et al.* (2017) and FAO (2017) reported that the rise in fish production in Nigeria covers only 55% of her total fish consumption (1,333,000 tonnes), while the remaining 45% was accounted for by importation. Meanwhile, FAO (2018), estimated that Nigeria would have a 36.2% increase in aquaculture production from 2016 to 2030. Although, catches from the inland waters shows Nigeria is enjoying an increased catch from 337,874 tonnes in 2015 to 377,632 tonnes in 2016 compared to 287,937 tonnes which was the average catch between 2005 and 2014, this indicates that Nigeria experienced a 31.2% increase in inland catch from the 2005 to 2014 average and 2016 catch while she had an 11.8% increase between 2015 and 2016 (FAO, 2018).

Increased demand for fish and fish products will mostly be met by growth in supply from aquaculture (FAO, 2016a). It is estimated that, for fish availability to meet projected demand globally, aquaculture production has to double by 2050 improving aquaculture that can lead to significant food security and developmental benefits (Waite *et al.*, 2014). Sub-Saharan Africa is said to have the fastest growing aquaculture industry (Waite *et al.*, 2014) and despite Nigeria being second leading aquaculture producer in Africa, having a strong aquaculture growth, contributing 0.4% of the world's total aquaculture production (FAO, 2018), aquaculture is the least exploited fishery sub-sector in Nigeria and this sub-sector is hampered by high operational costs, feed, low supply of quality fish seed, lack of steady power supply and increased competition from cheaper imported farmed fish and fish products amongst other constraints (Omitoyin, 2007; Waite *et al.*, 2014; Ozigbo *et al.*, 2014; Ejiola and Yinka, 2012). Having reported that, carp, catfish and tilapia among other freshwater species, will account for most of the growth in aquaculture production

and will represent over half of the total aquaculture production by the year 2025 (FAO, 2016a), the steadily increasing importance of fish farming in Nigeria has birthed improvements in the technologies necessary for the initial requirements for productive aquaculture. Also, Nigeria among other African countries, is said to be positioned for high aquaculture growth in *Clarias gariepinus* and *Oreochromis niloticuss* production (Waite *et al.*, 2014). However, Nigeria still remains a single species (catfish) producer. Catfish, usually grown in ponds and tanks is the most farmed species, contributing over half of the total aquaculture production by volume in Nigeria.

A survey of culturable fish seeds across Nigeria showed that there are potentially viable culturable fish species indigenous to every region that are of aquaculture potential (Ngueku, 2015). As a result of urbanization and increased rural-urban migration, fish consumption continues to grow in Sub-Saharan African countries. Therefore, an increase in fish productivity and diversity will lead to higher food and nutrition security especially from aquaculture development with an assurance of a variety of fish availability for the teeming Nigerian population and ensuring food security in families (Waite *et al.*, 2014). Despite reports of other potential aquaculture candidates introduced in Nigeria in the past, particularly carps, have flourished in the nation, since carp culture requires more complex procedures and more ponds than tilapia culture. (Akankali *et al.*, 2011). 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).

According to FAO (2014), tilapia ranks as the number two most cultured species after the carps (FAO, 2008) surpassing the salmonids group and have become globally important aquatic species in many countries worldwide. The farming of tilapia is the common extensive type of aquaculture worldwide (FAO, 2014). Worldwide, *Oreochromis niloticuss* production increased during the years 2001 to 2006 from 1,113,737 mt to 1,988,726 mt representing a growth of 79% with production reaching two million tonnes in 2007 covering about 83% of the total production for the tilapia group (FAO, 2008),

with a production of 7.9 million tonnes in 2016 (FAO, 2018) thus, making it one of the fastest growing fresh- and brackish water fish aquaculture productions, respectively. However, tilapia culture in Nigeria remained largely a subsistence level (Miller and Atanda, 2011). Between 2000 and 2005, the tilapia aquaculture industry based solely on *O. niloticuss*, cultivated under different farming systems; commercial and artisanal production systems, produced 14,388 tonnes to 19,546 tonnes (Elekwachi, 2018). This industry is however, experiencing growth, albeit a slow one as has been reported (NBS, 2017). In Africa, Nigeria is the second largest producer of cultured tilapias after Egypt (Fagbenro *et al.,* 2010). Tilapia culture in Nigeria is characterized mainly by subsistence activity until recently, when it began to develop fast following the successful commercial farming of catfishes during the last decade (Miller and Atanda, 2011; Okwodu, 2016). Intensification of tilapia farming has been promoted and farmers are enhancing growing condition of fish (Asche, 2008). The most obvious result of successful aquaculture is an increase in the amount of fish available for human consumption, either locally or worldwide (Brummett *et al.*, 2008).

Oreochromis niloticuss is the most famous and subjected to several researches of all tilapia species, this is influenced by its economic importance (Elekwachi, 2018). Compared to other tilapia species, *O. niloticuss* is most common; this is due to its tremendous growth. *Oreochromis niloticus* is a desirable fish for cultivation because of its ease of reproduction, quick growth, strong feed conversion, acceptable flavor and customer acceptability, high tolerance to unfavorable environmental conditions, and disease resistance.(Chakraborty and Banerjee, 2009). Despite having many good culture qualities, one of the major problems in commercial tilapia production is its precocious maturity and the following uninhibited reproduction, leading to increased competition for feed followed by stunted growth, small size and low commercial/market value (Pradeep *et al.*, 2011; Omitoyin *et al.,* 2013).

In tilapia populations, male species grow faster and are more even in size than female counterparts (Chakraborty and Banerjee, 2009; Megbowon and Fasina-Bombatta, 2010; Nwachi and Yuzine, 2016), bringing about preference for single sex populations of only males. Monosex production system exhibits several advantages over mixed sex

production systems; reduction or total elimination of unwanted reproduction, faster growth, preventing energy diversions into gonad production and courtship behavior leading to larger uniformity of size at harvest (Cnaani and Levavi-sivan, 2009; Kamaruzzaman *et al.,* 2009; Rajaee, 2011; Shamsuddin *et al.,* 2012) hence, proving to be the best way to rapidly increase yields of this important food fish (Sun *et al.*, 2014).

Several methods of producing monosex population include; manual sexing, hybridization and hormonal sex reversal. Manual sexing of tilapia entails manually sorting and separating females based on sexual dimorphism spotted in the urogenital papilla from the males as reported by Nwachi and Yuzine (2016). Tilapia are in general, difficult to sex as differentiating between males and females can be challenging before attaining a certain size, at least 25g. Though this method is simple, it is time consuming, labour intensive, requires expert/qualified personnel and poses other unforeseen problems as opined by Nwachi and Yuzine (2016). Hybridization (crosses) between some cichlid species such as Nile tilapia and the blue tilapia results in the production of predominantly male offspring approaching 100% (El-Zaeem and Salaam, 2013; Martinez *et al.*, 2014). However, for most freshwater aquaculture of the several cichlid species, *O. niloticus* is the most prominent species of choice due to its high growth potential (Yosef, 2009; Fuentes-Silva *et al.*, 2013).

In the face of these developments, hybridization did not successfully solve the problem of unwanted spawning mainly due to difficulty in maintaining production of all-male hybrid populations. Ferdous and Ali (2011), stated that hormonal sex reversal is a method of altering sexes of fish from one sex to another by administering synthetic steroidal hormones before or during the stage of sexual differentiation where the first feeding fry are fed male hormone or androgens thereby, masculinizing the fish. At the time of hatching tilapia larvae are still sexually undeveloped, therefore, in the early period of gonadal differentiation, alteration in sex hormone levels can affect and possibly change the final sex regardless of the genetic sex (Ferdous and Ali, 2011; Sukmanomon *et al.,* 2012).

Masculinization of tilapia for the production of all-male populations continues to be an important tool for fish farmers to prevent unwanted reproduction that lead to fish with

larger growth potential but the use of synthetic hormones have been clouded with criticisms by consumers based on the fact that many believe the residual effects of such hormone has harmful side effects (El-Sayed *et al.,* 2012). This has led to the need to explore other alternatives for sex reversal agents from non-synthetic origin. The demand for organic fish is rapidly increasing and supply very inadequate (Ozigbo *et al.*, 2014), phytogenic extracts are a fast way to achieving ecologically and economically acceptable fish. A number of medicinal plants have been used by man for so many reasons, since the beginning of human civilization. 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 will be widely accepted(Ekor, 2013). Among the alternatives which can be considered to mitigate the problem of using synthetic steroid for monosex tilapia production is the use of *Tribulus terrestris*extracts as a potential substitute for synthetic Methyltestosterone.

Numerous medicinal herbal extracts in forms of crude, semi-purified and purified extracts have been reported to promote growth and feed utilization parameters as well as survival among tilapia species such as; evidences of improved antioxidant, enzyme activities and lipid profile of GIFT-tilapia challenged by *Streptococcus iniae* after fed diets containing *Aloe vera*(Gabriel *et al.*,2015a); assessing the growth and gonadal maturity as well as resistance to *Streptococcus agalactiae* infection following treatments by feeding some crude plant extracts to *O. niloticuss*(Kareem *et al.*, 2016); assessing thegrowth and nutrient utilization as well as entire body composition of *Oreochromis niloticuss* administered fish diets that contained caraway seed meal as a feed additives (Ahmad and Abdel-Tawwab, 2011), just to mention a few. In the same view, dietary *Tribulus terrestris* extracts could also be investigated whether it improves growth, immune activity and survival of Nile tilapia.It is pertinent to state that plant extracts vary different1y based on the chemical constituents of such plant. Solvents such as acetone, ethanol, ethyl acetate, petroleum ether, toluene and water with different polarities can be used to obtain extracts (Susmitha *et al.*, 2013). Hence, the types of plant extracts (i.e. crude from whole or parts, or compounds from whole or parts of the plant), and extraction methods remain crucial aspects to consider in phytomedicinal studies in aquaculture.

Female tilapia species are very fertile, mostly reproducing at an early age and small size leading to stunted physical growth. Their male counterparts on the other hand, show rapid growth hence, the preferred choice for monosex culture (El-Griesy and El-Gaman, 2012). Plant extracts containing diverse bioactive compounds such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils have been reported to reduce stress, promote growth, stimulate appetite, improve immunity in fish culture (Citarasu, 2010; Chakraborty and Hancz, 2011). Hence, the need to consider a potential alternative for inducing sex reversal in fish. However, there are significant variations regarding the efficacy of different phytochemicals for production of all-male fish population and the potential anabolizing and virilizing effects of such plant extracts needs to be clearly documented. Immersion as a method of extract delivery may also be useful in tilapia farming particularly for the purpose of sex reversal which uses fish larvae that are not fully developed to consume artificial feed.

Tribulus terrestris L. belonging to the family Zygophylaceae, is also called "Puncture vine". This is a plant with creeping branches with a semi-perennial underground stem and root system that is widely distributed in both tropical and warm temperate countries in Africa, Southern Europe and Western Asia, this plant grows as a summer annual plant in colder climates as well (Akram *et al.,* 2011).It is made up of different constituents such as; alkaloids, flavonoids and sapogenins, which have several properties like anti-inflammatory, immunomodulatory and anti-tumor activities. Flavonoids have been asserted to be very common among other compounds that occur naturally to possess anti-oxidant and hepato-protective elements (Rakesh *et al.*, 2009; Gültepe *et al.*, 2014). These phytochemical substances have a far-ranging biochemical reactions (Kavitha *et al.*, 2011).

Different forms of *Tribulus terrestris* is consumed by humans, supposedly for body and muscle building. This plant has also exhibited antioxidant and hepato-protective activities in *Oreochromis mossambicus* (Kavitha *et al.*, 2011) as well as exhibiting sex reversal properties in other species of fish (Cek *et al.*, 2007; Turan and Cek, 2007; Yeganeh *et al.*, 2017). This implies that *T. terrestris* extracts orally administered to Nile tilapia can

potentially improve the somatic growth indices, impact on their nutritional composition, many biological and chemical attributes as well as sex reverse larvae of this fish.

1.6 **Justification of the study**

Tilapia is quickly becoming the most preferred fish by consumers. To control the menace of tilapia overpopulation, predator species such as the *Clarias gariepinus* and *Heterobranchus longifilis* have been cultured alongside tilapia in Nigeria (Fagbenro, 2004) and plant extracts have been investigated and researched as fertility control agents in tilapia and their contraceptive efficacies in combating the problem of tilapia overpopulation in ponds have been established (Jegede, 2009; 2010). Even with these methods, the main drawback to tilapia culture still remains the excessive recruitment in ponds, which result in low yields of harvestable market size (Fagbenro *et al.*, 2010 and Dauda *et al.*, 2014).

Masculinization of tilapia as stated earlier, continues to be an important means for aquaculturists to prevent undesirable reproduction, which ultimately leads to higher fish growth, however, the popular means of all-male tilapia population via the use of synthetic hormones have been shrouded in criticisms by many stakeholders in the industry based on the believe that, the residual effects of such hormone has harmful side effects (El-Sayed *et al.,* 2012). This, among other factors, has led to the need to search for other alternatives to achieving sex reversal of fish from non-synthetic origin. Since time immemorial, several plants with medicinal properties have been exploited by man for a number of reasons. Thus, researches to use the natural occurring materials from these medicinal plants as additives to achieve improved animal performance, enhance feed utilization and efficiency as well as induce sex-reversal in fish have been on-going and is gaining wide acceptance (Ekor, 2013). Among the several alternatives which can be considered to mitigate against the use synthetic steroids for sex reversal of tilapia is *Tribulus terrestris* extracts explored as a potential sex reversal substitute for synthetic 17-α-methyltestosterone.

To justify these assertions, the demand for organic fish is rapidly increasing, while the supply is very inadequate (Ozigbo *et al.*, 2014). The requirements of the present market for ecological production laid the use of plant extracts in the feed industry and veterinary attendance as an alternative of nutritive antibiotics and synthetic hormones (Grigorova *et al.,* 2008). An alternate technique for commercial production of all-male fish populations

will be the use of phytogenic extracts. Phytogenic extracts such as *Mucuna pruriens, Asparagus racemosus*, *Basella alba*, *Tribulus terrestris*(Omitoyin *et al.*, 2013; Mukherjee *et al.*, 2015a; Ghosal *et al.,* 2016; Yeganeh *et al.*, 2017).

Tilapia can be masculinized by direct synthetic hormonal treatment that is efficient and straight forward, however, synthetic hormones are more expensive, their administration in fish is time-consuming and labour-intensive and this requires expertise. However, Asad*et al.*(2010) noted that one disadvantage of synthetic hormone treatment is the increased risks of long term exposure of workers handling the hormone during food preparation and feeding which may cause adverse effects on their health. Further, synthetic hormones accumulate in the sediment water and aquatic biota while having extremely adverse effect on living organisms (including other fish) at a very low concentration (Rezka *et al.*2015). There are also global concerns about consumer perceptions of eating hormone treated fish and its effects on the environment as evidenced in the reports of several researchers. Dergal *et al.* (2016) confirmed the authenticity of these concerns in their report while monitoring 17α-methyltestosterone residues in *Oreochromis niloticuss* flesh and experimental water after sex reversal while Mlalila *et al.*, (2015) discussed these concerns when they addressed safety of human food and environmental health regarding the use steroids in monosex tilapia production. In the same light, Megbowon and Mojeku (2014) summarized the effects of tilapia sex reversal on the fish, man and environment. Additionally, Waite *et al.* (2014) in addressing improved productivity and environmental performance in aquaculture stated that aquaculture can cause water pollution from discharge of waste water that contain hormones and other chemicals emphasizing that, it is difficult to control this pollution as these wastes are quickly dispersed into surrounding water bodies. With these hampering the easy production of all-male tilapia, there is an urgent need for a substitute for synthetic hormone in fish masculinization.

1.7 **Objectives**

The main objective of this research is to assess the sex reversal of *Oreochromis niloticuss* using differently processed *Tribulus terrestris* extracts.

The specific objectives are to:

- Determine the phytochemical composition of *T. terrestris* extracted with different solvents.
- Evaluate the effects of inclusion of *T. terrestris* extracts on growth parameters and nutrient utilization of *O. niloticuss.*
- Assess the effects of *T. terrestris* extracts on the haematology and serum biochemistry of *O. niloticuss*.
- Assess the sex reversal changes in *O. niloticuss* larvae fed with *T. terrestris* extracts.

1.8 Research hypotheses

$H0_1$: There is no significant difference in the phytochemical composition of *T. terrestris* extracted with different solvents.

$H0_2$: There is no significant difference in the growth parameters and nutrient utilization of *O. niloticuss* that were supplied feed with different inclusion rates of *T. terrestris* extracts

$H0_3$: There is no significant difference in the haematology and serum biochemistry of *O. niloticuss*fed feed with various *T. terrestris* extracts inclusion.

$H0_4$: There is no significant difference in the sex of *O. niloticuss*fed feed with *T. terrestris* extracts inclusion.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of fish in diets

Generally, people in developing countries consume a lot of staple foods which are often deficient in other essential nutrients but fish consumption will help in correcting the imbalance between calories and protein in these diets where even small quantities of fish can have a significant positive nutritional impact, given that it is a concentrated source of essential dietary components (WWF, 2016). It also provides fatty acids, vitamins and other vital nutrients like iodine and selenium, which do not exist in this quantity or variety in any other cereal or meat (Beveridge *et al.*, 2013; Kawarazuka and Béné 2011). Fish is frequently the only available and affordable source of animal protein in the coastal regions of developing countries. Hence, the World Health Organization (WHO) recommends the regular consumption of fish – one to two portions a week. With an average portion size of 150 g, this results in a worldwide recommended annual consumption of 11.7 kg fish per capita (WWF, 2016). Globally, fish and fish products provide an average of only about 34 calories per capita per day. However, their daily contribution can exceed 130 calories per capita in countries where alternative protein foods are lacking and where a preference for fish has developed (FAO 2018).Since, its accessibility can be made easier and economically less cumbersome, fish as animal protein can be readily made available to all. Fish and fishery products therefore represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health (FAO, 2012).

2.2 Status of Fish Production in Africa and Nigeria

Several authors have emphasized the great importance of fish in global food and nutrition security, claiming that it provides around 20% animal protein, fatty acids, and micronutrients to over 3.1 billionpeople worldwide. (WWF, 2016). Fish proves to be a

good source of high-quality protein and essential fatty acids leading to a surge in demand for high-quality fish (World Bank, 2013). Fisheries and Aquaculture make crucial contribution to the world's wellbeing and prosperity (FAO, 2012). Aquaculture has been reported to be the world's fastest growing food production sector (FAO, 2012). Since the mid-1990s, aquaculture has been the force driving growth in total fish production as capture production has leveled off and is on decline. Its contribution to world total fish production climbed steadily from 20.9% in 1995 to 32.4% in 2005 and 40.3% in 2010. For human consumption, aquaculture contribution reached 47% in 2010 as against only 9% in 1980(FAO, 2012). Since the early 1960s, the average annual increase in global food fish consumption has surpassed population growth and exceeded consumption of meat from all terrestrial animals combined, except poultry. In per capita terms, food fish consumption has grown from 9.0 kg in 1961 to 20.2 kg in 2015, at an average rate of about 1.5 percent each year (FAO, 2018).

In 2013, fish accounted for about 17 percent of animal protein intake worldwide and 6.7 percent of every protein consumed (FAO, 2016b). Due to the great importance in is consumption, its production must equally be regarded with utmost importance. Fish production has been growing steadily over the years at least at the global level. The emergent importance of fish protein for feeding an increasing world population is subjecting fisheries to increasing pressures so as to assure sustainable supplies of wild and cultured fish. Since the late 1980s, the aggregate global level of capture fisheries output has stagnated while, aquaculture has grown faster than all other major food sectors (Muir, 2013 and FAO, 2013). Food and Agriculture Organization (2012) predicted that the proportion of food fish derived from aquaculture will exceed that from capture fisheries by 2018. These reports have led to the conclusion that any increase in demand for fish can only be met by aquaculture (Hall *et al.*, 2011).According to Busari (2018), African governments under the tutelage of the African Union, identified the great potential of fish farming and are determined to encourage private sector investment, stating that, potential exists for fish farming to make a difference. He further reiterated that, the fish sector provides income for over 10 million people engaged in fish production, processing and trade in Africa.

Aquaculture development in Nigeria as well as Africa has been reported to be insignificant compared to Asia and Europe (Sogbesan and Ekundayo, 2014). Okwodu (2016) posited that, Nigeria produced half of Africa's fish, stating that only six countries account for 89% of Africa's total fish production. The growth, expansion and production of aquaculture in northern part of Africa especially, Egypt is more advanced in techniques and technicalities in comparison to the Sub Saharan regions. Aquacu1ture (fish farming) in most sub Saharan regions is stil1 essentially a loca1, supporting or occasiona1 activity happening in farms with fresh water p0nds that are small (Okwodu, 2016). Aquaculture development in most African countries is primarily focused on socio-economic objectives such as nutrition improvement in rural areas, income generation, diversification of farm activities (integrated farming) and creation of employment especially in rural communities where opportunities for aquaculture in northern part of Africa especially, Egypt is economic activities are limited. This approach over the years has resulted in sustained aquaculture growth in some African countries such as Côte d'Ivoire, Egypt, Ghana, Malawi, Nigeria and Zambia (Ayoola, 2010). While there is still room for enhancing aquaculture production in Africa through improved production systems, genetics and general farm management principles, the desired and expected growth of aquaculture to meet the ever-increasing demand for fish and satisfy its socioeconomic functions is only achievable through cost-effective and high quality fish feed.

In Africa, Nigeria is now one of the most significant and strongly growing producers in aquaculture. This is the result of growing population and high demand for fish which has positioned the country on a market-driven path (Elekwachi, 2018). In spite of the remarkable growth of aquaculture in Nigeria, it is a well-known fact that it is the largest importer of frozen fish in Africa (Atanda, 2012). In order to mitigate the ugly situation, aquaculture practices must be intensified. One sure way of ensuring improvement on fish production is by realigning interest in tilapia production, because it is a very important aquaculture species whose place has been supposedly occupied by catfish in Nigeria. However, the desirability of monosex populations for tilapia culture is well established (Dauda *et al.*, 2014). Nigeria has over 14 million hectares of inland water surface, out of which about 1.75 million are available and suitable for aquaculture (FAO, 2016a). In

Nigeria, aquaculture is predominantly an extensive land based system, practiced at subsistence levels in fresh waters (Busari, 2018). Commercial farming has yet to become widespread (Okwodu, 2016). At present, most fish farmers operate small-scale farms ranging from homestead concrete ponds (25 – 40 meters) to small earthen ponds (0.02 - 0.2 hectares).

The need arose from the decrease in supply from ocean fisheries as a result of over-fishing, habitat destruction and pollution. One of the ways to bridge the gap between the reduced fish supply and increased world food fish demand is through aquaculture (Ozigbo *et al.*, 2014). In Nigeria, aquaculture development has been driven by social and economic objectives, such as nutrition improvement in rural areas, generation of supplementary income, diversification of income activities, and the creation of employment. This is especially true in rural communities, where opportunities for economic activities are limited. Only in recent years has aquaculture been viewed as an activity likely to meet national shortfalls in fish supplies, thereby reducing fish imports (Adedeji, and Okocha, 2011). According to Ekunwe and Emokaro (2009) Statistics indicate that Nigeria is the largest African aquaculture producer, with production output of over 15,489 tonnes per annum, this is closely followed by Egypt with output of about 5,645 tonnes. Only five other countries: Zambia, Madagascar, Togo, Kenya and Sudan produce more than 1,000 tonnes each. Ekunwe and Emokaro (2009) further showed that Nigeria imports about 560,000 tonnes of fish estimated at about $400 million annually while annual domestic fish supply in Nigeria stands at about 400,000 tonnes. Further still, FAO estimated that Nigeria's fish production was 1,027,000 tonnes, adding that, the fisheries sector accounted for 0.5% of the national GDP in 2015 with 36%, 33% and 31% contributions from marine catches, inland water catches and aquaculture, respectively while Olaoye and Ojebiyi (2018) estimated that fisheries contributes 5.4% to Nigeria's GDP. However, as at 2017, total fish production in Nigeria was 1,027,058 tonnes (NBS, 2017) and the demand for fish was 3.32 million tonnes (FCWC, 2016) leaving a deficit of over 2 million tonnes. This increased demand for fish led to a higher amount of fish imports to about $1.2 billion in 2013 (FAO, 2015) and ₦125 billion importing 1.9 million metric tons fish in 2015, leading to Nigeria being classified as one of the largest fish importers (Olaoye and Ojebiyi, 2018).

2.3 Tilapia as aquaculture candidate for improved fish production

According to FAO (2016a), an increase in freshwater species such as carp, tilapia, and catfish will account for 60% of total aquaculture production in 2025, putting Nigeria in a strategic position to improve her fish production through aquaculture, especially since Nigeria is the world's largest producer of *Clarias gariepinus* (FAO, 2016b). Although, *Clarias gariepinus* remains the major fish cultured in sub-Saharan Africa, tilapia is quickly catching up even in Nigeria as many farmers and consumers are choosing to culture tilapia especially for export purposes (FCWC, 2018).

Oreochromis niloticuss is an African cichlid endemic to Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Egypt, Gambia, Ghana, Guinea, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo and Uganda and have been introduced to many other counries (Froese and Pauly, 2004; 2011). Nile tilapia has proven to be an important food fish that has been introduced to many different parts of the world by man with wide spread occurrence on all continents except Antarctica (Senanan and Bart, 2010).They are omnivorous by nature, but feed on a wide range of natural food organisms, such as plankton, planktonic and benthic aquatic invertebrates, larval fish, bacterial film and detritus (FAO, 2012).As purported by Makori *et al.* (2017), tilapia is a tolerant species, tolerant to high salinity, high water temperature, low dissolved oxygen, and high ammonia concentrations, compared to most of fresh water species.It is reported that this species can tolerate oxygen levels lower than 0.3 mg/l and grow well in temperatures between 25 ^{0}C and 35 ^{0}C with an optimum temperature range of 25 ^{0}C to 27 ^{0}C according to Makori *et al.* (2017).

Oreochromis niloticuss is a cichlid species which sexual maturity depends on age, size and environmental conditions and generally have early sexual maturity before reach market size (FAO, 2016b). Tilapia is susceptible to change in temperature as evidenced by their increased ratio of male to females as temperature rises with sex separation caused by 1ow temperatures(Fuentes–Si1va *et al.*, 2013). It is evidenced by researches that at the critica1 stage of sex differentiation, temperature is very important especially in very young fish (Baroiller *et al.*, 2009). Baroiller and D'Cotta (2016) posited that, at very high

temperatures during sex differentiation, obstruction of ar0matase that enhance the conversion of andr0gens to estr0gens happens which leads to a population having more males than females. This is evidenced in masculinizing tilapia at 32 ^{0}C. Growth of fish significantly increases with increasing dietary protein (cp) up to 46 % where an optimum growth and feed utilization of *Oreochromis niloticuss* is obtained (Ahmad and Abdel-Tawwab, 2011). The digestible protein requirement for Nile tilapia in the sexual reversion phase is 38.6 % where the growth was maximum and the survival decreased linearly as digestible protein levels increased (Ahmad and Abdel-Tawwab, 2011).

2.4 **Sex determination in fish**

Controlling the sex ratio is crucial in aquaculture as a balanced sex ratio is a good management tool for broodstock management and fish seed production. However, the production of monosex populations is highly desirable in some species due to the existence of sexual dimorphism in this species, primarily in growth or sexual maturation. Gonadal development are all developmental processes directed at transforming an undifferentiated primordial gonadal cell into a mature ovary or testis (Martinez *et al.*, 2014). The sex of gonads is fundamentally determined by either genetic or environmental processes operating from the commencement of development where a binary selection is made related to the fate of the undifferentiated primordial cell. According to Martinez *et al.* (2014), major events leading to ovarian or testicular differentiation in fish starts with sex determination where gender of the fish is established and can be activated by the action of a maj0r sex deciding master gene, several sex-associated loci, an environmental factors such as temperature occurring normally in the habitat of the species or a combination of all these generally, when these gonads are still sexually undifferentiated or even before they are formed at the pre-gonadal stage.

2.5 **Chemical Constituents of medicinal plants**

Phytochemicals are non-nutritive components that have protective or disease preventive properties found in plant-based diets (Arendt and Zannini, 2013). They are non-essential

nutrients; they are not required by the human body for sustaining life. It is well-known that plant produce these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. Phytochemicals are naturally occurring in all parts of medicinal plants that have defense mechanism to protect humans and animals from various ailments and these phytochemicals are primary and secondary compounds in plants (Ahmad*et al.*, 2016). Phytochemicals have been broadly classified into six major categories based on their chemical structures and characteristics; alkaloids and other nitrogen containing compounds, carbohydrate, lipids, phenolics, terpenoids (Huang *et al.*, 2016).

Primary compounds of plants are chlorophyll, proteins and common sugars while secondary compounds are phenolic compounds such as flavonoids, tannins, and lignins, terpenoid, and alkaloids (Wadood *et al.*, 2013) many of which act as antioxidants. Flavonoids make up the largest class of phytochemicals of these phenolic compounds (Du *et al.*, 2016). Flavonoids are molecules with a low molecular weight. Flavonoids include flavones, isoflavones, flavonoids, flavonols, flavanones, anthocyanins, and proanthocyanidins, according to the flavonoid categorization system (Altemini *et al.*, 2017). Terpenoids exhibit various important pharmacological properties while Alkaloids are used as anaesthetic agents and are found in medicinal plants (Wadood *et al.*, 2013). Tannins are natural products found in many plant families and have large amounts of phenolic rings in the structure (Altemini *et al.*, 2017). Saponins are a diverse group of compounds that is diverse in the plant kingdom having varying structures, physiological and biological effects (Addisu and Assef, 2016). Saponins in herbs have been found to be in very low levels to have any harmful effects when consumed (Francis *et al.*, 2003). Odufuwa *et al.*, (2013) further reported that saponins act as both anti-nutrient and antioxidant in humans stating that saponin in cultivated plants are terpenoid saponin while those in plants not cultivated (herbal) are steroidal saponins.

Altemini *et al.*, (2017) reported about 20% of known plants have been used in parts or wholly in pharmaceutical studies, leading to positive impacts on the healthcare system such as treating tumours and other harmful diseases and may supplement the needs of the human body by acting as natural antioxidants to protect cells against oxidative damages

(Odufuwa *et al.*, 2013). Others have hormonal actions and stimulate enzymes while others have antibacterial effects (Lillehoj *et al.*, 2018).

2.6 **Methods of phytochemical extraction**

Extraction is a process of separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures Handa *et al.* (2008). He further stated that, products obtained from plants during extraction are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, semi-solid extracts and powdered extracts (Handa *et al.*, 2008). Plants extraction is an experimental activity as different solvents are utilized at varying conditions such as temperature, duration (Ingle *et al.*, 2017) and method of extraction.

According to Pandey and Tripathi (2014), some of general methods of medicinal plant extraction include maceration, percolation, decoction, hot continuous extraction (Soxhlet), supercritical fluid extraction, microwave-assisted extraction, and ultrasound extraction (sonication).

3.6.1 **Maceration**

Pulverized whole or part of plant or coarsely powdered material is allowed to contact with the solvent which is in a stoppered container and allowed to stand at room temperature for a particular time period (at least three days) with frequent agitation until the soluble matter has dissolved (De Silva *et al.*, 2017; Saxena *et al.*, 2013; Handa *et al.*, 2008). At the end of the process the solvent is drained off and the marc is pressed or centrifuged and the combined liquids are clarified by filtration or decantation after standing (Tiwari *et al.*, 2011). Maceration is a very simple extraction technique and can be used for thermolabile constituents however, it is disadvantaged by long extraction duration with low extraction efficiency since active ingredients cannot be totally

extracted (Zhang *et al.*, 2018; De Silva *et al.*, 2017). Digestion is a form of maceration in which gentle heat is used during the process of extraction when moderately elevated temperature is not objectionable (Handa *et al.*, 2008).

Many researchers have reported on the use of maceration as an extraction method relating to its efficiency, cost and suitability. Ćujić *et al.* (2016) suggested that maceration was an effective method of phenolic compounds extraction from chokeberry fruit when high yields of this compounds were achieved following extraction by maceration. Similarly, Vongsak *et al.* (2013) reported that maceration with 70% ethanol produced the highest phenolics and flavonoid contents in *Moringa oliefera* compared to soxhlet extraction and percolation using the same solvent. Evaluation of polyphenols from *Serpylli herba* by Jovanović *et al.* (2017) revealed that, there was statistically, no significant variation in quantity of total flavonoid obtained between maceration and heat assisted extractions. Zhang *et al.* (2018) asserted that there are economic benefits to using maceration compared to microwave-assisted extraction from the reports of Albuquerque *et al.* (2017) where microwave-assisted extraction of catechin from *Arbutus unedo* fruits was most effective, though, a lower temperature was utilized in maceration which produced an identical extraction yields. However, Jin *et al.* (2011) concluded that maceration extraction against microwave-assisted method, reflux extraction, ultrasound-assisted extraction had the lowest extraction efficiency of *Cajanus cajan* leaves.

3.6.2 **Percolation**

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts (Handa *et al.*, 2008). A percolator which has a narrow cone shaped vessel open at both ends is used for this technique (Majekodunmi, 2015) where plant materials are moistened with an appropriate amount of the specified solvent and allowed to stand for a period of time in a well closed container or percolation chamber (De Silva *et al.*, 2017). Additional solvent is added to form a layer above the plant material and is rinsed with the solvent for several times until the active ingredient is extracted or allowed to macerate in the closed percolator for a duration of 24 hours (De Silva *et al.*, 2017).

Percolation is more efficient than maceration (Zhang *et al.*, 2018). Compared to decoction/reflux as extraction method, Zhang *et al.* (2014) concluded that there was no significant difference between percolation and refluxing extractions of *Undaria pinnatifida* despite percolation producing higher contents of fucoxanthin.

3.6.3 **Decoction**

In this process of extraction, the plant material is boiled in a specified volume of water for a defined duration after which it is cooled and filtered (Handa *et al.*, 2008), these extracts are usually laden with large amounts of water soluble impurities (Zhang *et al.*, 2018). This method is suitable for extracting, heat-stable compounds and hard plants materials that are water-soluble but cannot be used for the extraction of volatile compounds (Zhang *et al.*, 2018; Pandey and Tripathi, 2014).

Zhang *et al.* (2013) suggested that decoction process might enhance the dissolution of some bioactive compounds in two traditional Chinese medicines, *Sanhuang Xiexin Tang* and *Fuzi Xiexin Tang* compared with the maceration process when the researchers monitored 17 active constituents in these traditional Chinese medicines.

3.6.4 **Soxhlet (Hot Continuous) Extraction**

Soxhlet extraction is an automatic continuous extraction method with high extraction efficiency that requires less time and solvent consumption than maceration or percolation especially when the desired compound has a limited solubility in a particular solvent and impurities are less soluble in the solvent (Zhang *et al.*, 2018; De Silva *et al.*, 2017). Finely ground sample is placed in a porous bag and the solvent in which the desired compounds are going to extracted is kept in the round bottom flask (Azwanida, 2015). This process is continuous and carried out until a drop of solvent from the siphon tube does not leave residue when evaporated (Handa*et al.*, 2008).

Adhikari *et al.*, 2018 reported that extraction yield was highest in stem soxhlet extraction of *Taxus wallichiana* using ethanol as solvent, while being at par with acetone and methanol as solvents against maceration as an extraction method using the same solvents.

This is in tandem with Hossain *et al.*, (2013) who concluded that the extraction of *Azadirachta indica* leaf powder in methanol using soxhlet extraction produced more phytochemicals even non polar compounds compared to other methods. However, Soxhlet extraction of *Moringa oliefera* leaves resulted in lower yield, phenols and flavonoids content against a higher yield using maceration (Vongsak *et al.*, 2013).

In the light of the advantages of this extraction method compared to maceration and percolation methods being more economical as it relates to time and energy consumption and the overall financial inputs, the high temperature and long extraction time in the Soxhlet extraction can increase the chances of thermal degradation. This assertion is buttressed by the degradation of catechins in tea observed after Soxhlet extraction as well as the decreased concentrations of both total polyphenols and alkaloids from Soxhlet extraction method at 70 ^{0}C compared to those from maceration at below 40 ^{0}C (Chin *et al.*, 2013). Similarly, Anuradha *et al*. (2010) after removing lipodial material from *Clitorea ternate* flowers using soxhlet extraction reported that, anthocyanin, the major component of *Clitorea ternate* flowers was absent, suggesting oxidation and degradation had occurred. This method therefore, cannot be used for thermolabile compounds as prolonged heating may lead to degradation of these compounds.

3.6.5 **Supercritical Fluid Extraction**

Supercritical fluid extraction uses supercritical fluid as the extraction solvent involving the use of gases and compressing them into a dense liquid which is then pumped through a cylinder containing the material to be extracted and from there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas at lowered temperature and pressure and the gas is recovered for re-use (Ingle *et al.*, 2017; Prabu *et al.*, 2013). These are fluids with similar solubility and diffusivity to liquids and gases respectively and can dissolve a wide range of natural products (Zhang *et al.*, 2018). Supercritical gases used to extract active ingredients include; carbon dioxide, nitrogen, methane, ethylene, propane, ammonia among other gases (De Silva *et al.*, 2017).

Due to its inertness and non-toxicity, low critical temperature, low cost, and ability to extract thermally labile compounds, supercritical carbon dioxide (sCO_2) was widely used in supercritical fluid extraction (Zhang et al., 2018). However, argon is now being used instead of carbon dioxide because it is less expensive and more inert. The use of supercritical carbon dioxide in extracting essential oils from *Rosmarinus officinalis* and vinblastine from *Catharanthus roseus* were reported to have yielded higher than other methods by Conde-Hernández *et al*. (2017); Falcão *et al*. (2017), respectively.

Supercritical fluid extraction finds extensive application in the extraction of natural products, environmental samples and foods, essential oils and fragrances, pesticides and polymers with the advantage of leaving no solvent residue since sCO_2 evapourates absolutely (Patil and Shettigar, 2010; Handa *et al.*, 2008).

3.6.6 **Microwave assisted extraction (MAE)**

Microwaves generate heat by interacting with polar compounds and some organic components in the plant matrix using the ionic conduction and dipole rotation mechanisms (Zhang *et al.*, 2018). Simply, microwave assisted extraction combines microwaves with traditional solvent extractions (Ingle *et al.*, 2017). Microwave radiation acts together with dipoles of polar and polarizable materials causes heating near the surface of the materials and this energy is transferred by conduction making it a selective method that favour polar molecules and solvents with high dielectric constant (Azwanida, 2015). There are two types of microwaves assisted extraction methods, solvent extraction used for non-volatile compounds and solvent-free extraction used for volatile compounds (Vinatoru *et al.*, 2017). One significant advantage of this method of extraction is the decrease in thermal degradation and selective heating of plant materials (Zhang *et al.*, 2018).

Studies by Puttarak and Panichayupakaranant (2013) revealed that microwave assisted extraction yielded twice as much as soxhlet extraction of triterpene from *Centella asiatica*. Similar trends were also observed by *Xiong et al.*, (2016); Kumoro and Hartati (2015) in the extraction of lotus plumule *Nelumbo nucifera* and*Dioscorea hispida*,

respectively. Following the report *Li et al.* (2012) that microwave assisted extracts produced more antioxidant activity and yielded more phenolic contents than conventional methods using different solvents, *Altemini et al.* (2017) agreed with *Ballard et al.* (2010) that the main use of microwave assisted extraction is to extract antioxidants from plant with heated solvent under a lesser duration.

3.6.7 **Ultrasound Extraction (Sonication)**

The procedure is also called sonication, ultrasonic-assisted or ultrasonic extraction and involves the use of ultrasound wave energy with frequencies between 20 kHz to 2000 kHz for extraction this increases the permeability of cell walls and produces cavitation (Zhang *et al.*, 2018; Handa *et al.*, 2008). Ultrasound in the solvent producing cavitation accelerates the dissolution and diffusion of the solute as well as the heat transfer, which improves the extraction efficiency. The mechanic effect of acoustic cavitation from the ultrasound increases the surface contact between solvents and samples and permeability of cell walls (Dhanani *et al.*, 2013).While sonication is advantageous in low solvent and energy consumption and the reduction of extraction temperature and time as well as its extensive use in the extraction of thermolabile compounds, its disadvantage is having destructive effect of ultrasound energy on the active constituents of plants through formation of free radicals (Chemat *et al.*, 2017).

Researches on the extraction of two traditional Chinese medicines *Panacis Japonici Rhizoma* and *Dichroae radix* using sonication and reflux methods were not significantly different as both methods produced high extract yield and were time saving (Wu *et al.*, 2017; Guo *et al.*, 2015). Conversely, compared to heat assisted extraction and maceration methods, Jovanović *et al.* (2017), reported a higher yield of polyphenols from *Thymus serpyllum* using sonication with 50% ethanol as solvent. Similarly, based on high yield, selectivity and short extraction time, sonication was revealed to be an efficient method of extraction of *propolis* (Azwanida, 2015). Ebrahim *et al.* (2014); Yingngam *et al.* (2014) both reported higher efficiency of sonication in the extractions of anthocyanin from flower parts and phenols from *Cratoxylum formosum* respectively.

2.7 **Solvents for phytochemicals extraction**

Zhang *et al.*, (2018); Zhang (2015) emphasized the importance of selection of solvent for extraction stating that, selectivity, solubility, cost and safety are considered in the choice of solvents in addition to the fact that, extraction solvents affect the chemical composition and bioactivity of plant extracts. Selection of solvents for extraction depends on the solubility of the desired components of the material. Over time, solvents used for extraction of plants and plants products have varied according to the compounds of interest in these plants and the polarity of the solvents following the law of similarity and intermiscibility, moreover, the phytochemical composition of a plant extract relies highly on the polarity of solvent, plant sample to solvent ratio, particle size, temperature or heat and extraction method (Zhang *et al.*, 2018). An ideal solvent dissolves the desired compound, leaving the other constituents, other properties of ideal solvents for extraction as itemized by Pandey and Tripathi (2014) are; low toxicity, ease of evapouration, possession of preservative actions, promote rapid absorption of extract and inability to cause dissociation of the extract.

One important factor that affects extraction efficiency of bioactive compounds from plant materials is the extraction solvent (Ngo *et al.*, 2017). Different solvents have been used for extraction of different plant parts. Solvents used for extraction of plant biomolecules are selected based on the polarity of the targeted solute, meaning, highly polar solvents are used to extract hydrophilic or polar compounds while mixtures of low and high-polar solvents are more effective when target compounds with different polarities are to be extracted from plant material (Khan *et al.*, 2018; Altemini *et al.*, 2017). After extraction, the end product will usually contain residual solvents, it is pertinent to know the toxicity of the solvents used for extraction (Tiwari *et al.*, 2011).

Many researchers have deferred to water for the extraction and isolation of polar compounds especially those with antimicrobial activities seeing as it is dubbed a universal solvent (Tiwari *et al.*, 2011). Most traditional herbal or plants extracts primarily are aqueous extracts, but plant extracts from organic solvents were revealed to have higher antimicrobial activity compared to aqueous extract (Tiwari *et al.*, 2011; Das *et al.*,

2010). In some cases, for effective extraction, some modification is necessary for water as extraction solvent for example, extraction of alkaloids from plants where water is acidified as against using basic water for extraction of phenols.

Felhi *et al.* (2017) explained that lower content of phenolic compounds in acetone extracts of *Ecballium elaterium* compared to methanol extract may be explained by the low solubility of polyphenols in acetone due to hydrogen bond force between polyphenols and proteins. While the connection between tannin contents and extraction solvents can be linked to the polymerization degree for the tannins extracted by different solvents (Naima *et al.*, 2015), this was further asserted by Felhi *et al.* (2017) stating that the interactive abilities of solvent and tannins compounds are probably related to chemical compositions and structures. The solubility of the tannins is correlated to the degree of polymerization due to the increase in the number of hydroxyl groups. Arya *et al.* (2012) reported highest extraction yield of alkaloids, saponins, carbohydrates, tannins and flavonoids in *Psidium guajava* leaves using ethanol and hydro-alcohol extracts compared to compared to petroleum ether, chloroform and water suggesting that, phytochemicals in *P. guajava* are more soluble in polar solvents as the non-polar solvents showed no active compounds in the extracts.

Based on total phenols and flavonoids, Sulaiman*et al.* (2011) reported that 70% acetone was most efficient in the extraction of *Portucala oleracea* and 70% methanol for *Cosmos caudatus* using maceration methods. Lipodial materials were removed from powdered *Clitorea ternate* flowers using petroleum ether at 60 ^{0}C – 80 ^{0}C using Soxhlet extraction and further extraction of the marc with ethanol ascertained the presence of alkaloids and saponins (Anuradha *et al.*, 2010). Dhanani *et al.* (2013) emphasized the importance of solvent choice for extraction, when sonication of *Withania somnifera* by water as solvent reportedly produced maximum yield, compared to ethanol and water-ethanol at different extraction duration. Adhikari *et al.*, (2018) concluded that the higher extract yield with polar solvents suggested the presence of more polar molecules in *Taxus wallichiana* after extraction with acetone, chloroform, dichloromethane, ethanol, ethyl acetate, methanol and petroleum ether using soxhlet extraction and maceration. Further stating that low extract yield produced in ethyl acetate extracts compared to other polar solvents can be

associated to poor dielectric constant. Hepsibah and Jothi, (2017) reported that alkaloids and flavonoids were present in methanol, acetone, ethyl acetate and ethanol extracts of *Ormocarpum cochinchinense*, adding that saponins, phenols and tannins were only present in ethyl acetate, acetone, ethanol and methanol extracts of the plant.

The use of alcohols has ethanol is the preferred solvent for food grade extracts since methanol is toxic. The higher activity of the ethanol extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts (Tiwari *et al.*, 2011). Bimakr (2011) reported that increasing the polarity of ethanol by dilution with water up to 30% produced a higher concentration of flavonoid compounds compared to absolute ethanol.

2.8 **Evidence of phytochemicals as growth promoters in fish**

Several researches abound on the use of phytochemicals as growth promoters in animals and in fish, this is against the backdrop of the constant need to improve productivity especially in aquaculture. Though the plants 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 immunostimulating actions of these plant chemicals can enhance fish growth, however, the presence of anti nutirtional factors in these plants have limited the use of these sources in fish feed (Chakraborty, 2017). These assertions are evident in the reports of several studies.

A study of the effects of dietary *Aegle marmelos*, *Cynodon dactylon*, *Withania somnifera* and *Zingiber officinale* on growth of *Oreochromis mossambicus* carried out by Immanuel *et al.* (2009), revealed that, supplementing diets with acetone extracts of *C. dactylon*, *A. marmelos*, *W. somnifera* and *Z. officinale* and fed to *O. mossambicus* for 45days at 5% body weight had higher body mass of up to 139% stating that, the specific growth rate of fish fed these medicinal plant extracts were higher than those fed control diets without

plant extracts. Also, using a commercial mixture of phytoestrogens for *C. gariepinus* through immersion, a resultant increase in specific growth rate and total protein was observed by Yilmaz *et al.* (2009) as phytoestrogen levels increased while Abdel-Tawwab *et al.* (2010) recorded enhanced growth in *O. niloticuss* that were fed *Camellia sinensis* leaves at 0.5 g/kg for 12 weeks, further reporting that, the feed conversion/ratio with protein levels of the fish were enhanced as well. Dried powder of *Phyllanthus niruri* and *A. vera* gel were incorporated into a 37.5% C.P feed and fed to *Carassius auratus*for 60 days to determine the fish growth and survival and reported that *P. nururi* at 1.5% yielded the highest weight gain of 1.77g followed by *A. vera* at 1.0% with a weight gain of 1.4g while highest survival (80% and 70%) were recorded in fish fed with *A. vera* at 1.5% and *P. nuriri* at 1.5% respectively (Ahilan *et al.*, 2010).

In likewise manner, Ahmad and Abdel-Tawwab (2011) assessed the performance of growth, utilization of feed and the body-composition of *Oreochromis niloticuss* using caraway seed meal *Carum carvi* seed meal as feed additive. They fed *O. niloticuss* with an iso-nitrogenous and iso-caloric feed containing *C. carvi* seed meal in concentrations of 0.0 g/kg, 5 g/kg, 10 g/kg, 15 g/kg, or 20 g/kg for 12 weeks. They reported that the *C. carvi* supplemented diets produced better growth performance of the fish over the control group stating that the highest growths were recorded in fish fed with 10 g/kg while concluding that dietary supplementation with *C. carvi* seed meal was optimum for *O. niloticuss* at 12.5 g/kg based on second-order polynomial regression analysis of growth parameters. *Phoenix dactylifera* seeds were used as additive to improve feed utilization of *C. gariepinus* by Sotolu *et al.* (2014). The authors added *Phoenix dactylifera* seeds to *C. gariepinus* diets at 0%, 0.5%, 1.0%. 1.5% and 2% and fed for 70 days. They reported higher mean weight gain, and specific growth rate as well as protein efficiency ratio in fish fed with feed supplemented at 1.5%. Similarly, Omar *et al.* (2014) also fed *O. niloticuss* diets containing ginseng extracts at 0.2 g/kg and 0.4 g/kg and date palm extract at 3 g/kg and 6 g/kg to determine the effects of these natural phytochemical dietary supplementations on the growth response and nutrient utilization of *O. niloticuss* as well as the body composition of the fish. The authors found out at the termination of the research that, fish administered diets supplemented with phytochemicals had higher growths in terms of final body

weight and specific growth rates compared to the control group that were fed the basal diets. In conclusion, these authors stated that, dietary supplementation of fish feed with ginseng and date palm extracts can enhance the growth performance of *O. niloticuss*. Golestan *et al.* (2015) evaluated the growth of *Oncorhynchus mykiss* fed 48.25% crude protein diets supplemented with *Aloe vera* gel at 0.5 g/kg, 1.0 g/kg and 2.0 g/kg for eight weeks. The authors recorded the highest weight gain of 4.49 g in fish fed diets of 1.0 g/kg but stated that supplementation with *A. vera* gel had no significant difference in the growths of *O. mykiss*.

Chakraborty, (2017) reported the weight gain in *O. niloticuss*fed with varying concentrations of *B. alba* and reported that, though the fish fed with 17α-methyltestosterone had the highest growth, those fed with *B. alba* were significantly higher than the control. The author stated that a weight gain of 147.2g was achieved with a specific growth rate of 7.6% after 120 days of treatment. Hybrid red tilapia was fed herbal mixtures of *Vitex trifolia, Strobilanthes crispus* and *Aloe vera* by Manaf *et al.* (2016) to evaluate the growth performance of this fish. Methanol extracts of *V. trifolia, S. cripus* and *A. vera* were combined and supplemented in diets at 3.5 g/kg each for *Oreochromis* sp. for 60 days. They recorded improved growth in fish fed the herbal mixtures compared to those fed diets with no extract supplements with those fed *A. vera* and *V. trifolia* extracts showing the best growths. Essential oils from *Foeniculum vulgare* and *Allium sativum* were investigated by Hassan and Soltan (2016) for their growth effects on *O. niloticuss* when they used the oils as additives separately and in combination with *Bacillus licheniformis* to feed the fish for 84 days. At the end of the experiment, these authors reported that highest weigh gain and specific growth rates were recorded in fish fed diets containing these essential oils in combination with *Bacillus licheniformis*, however, there were no significant differences with those fed diets containing the oils separately. Assessment of the actions of four comestible crude plant extracts on the growth of Nile tilapia was carried out by Kareem *et al.* (2016). These authors assessed the effects of dietary extracts of *Azadirachta indica*, *Carica papaya*, *Cinnamomum camphora* and *Euphorbia hirta* on growth of *O. niloticuss* which were fed diets with these extracts at 2 g/kg for 90 days. They reported that, these extracts improved

the growth of fish except *A. indica* which was not significantly different from the control group.

Adenigba *et al.* (2017); Nian *et al.* (2017) both tested growth effects pollens of *Pinus tabulaeformis* would have on *C. gariepinus* and *O. niloticuss*. They reported that these pollens have a concentration dependent effect on these species as there were increase in weight gain and specific growth rates. The effects of *Phoenix dactylifera* seed extract on the growth of *Cyprinus carpio*was assessed by Mohammadi *et al.* (2018) where they fed *C. carpio* with diets supplemented with *P. dactylifera* seeds extracted by maceration with methanol at 0.0%, 0.5%, 1.0%, 2.0% and 4.0% at 3% body weight for 60 days. These researchers observed that, though there was a higher weight gain in fish fed at 0.5% supplementation, growth began to decline with increase in *P. dactylifera* extracts and concluded that, adding 0.5% of *P. dactylifera* extract can improve the growth of *C. carpio.* Similarly Kamali-Sanzigh *et al.* (2018) used *P. dactylifera* waste meal to substitute plant source by 5%, 10% and 15% in *C. carpio* diets and fed for 12 weeks and reported that, *C. carpio* body mass and SGR improved at fish given 10% and concluded also, that, plant sources in diets of *C. carpio* can be substituted up to 10% with *P. dactylifera* waste meal. *Urtica dioic* extracts however produced a decline in growths of *C. nigrofaciatum* immersed in water containing 200 mg/L and 300 mg/L (Babahajiani *et al.*, 2018). Gabriel *et al.* (2019) supplemented *C. gariepinus* diet with *A. vera* polysaccharide at 0.5%, 1.0%, 2.0% and 4.0% per kilogram for 8 weeks. These authors, contrary to previous reports on *A. vera*, reported that *C. gariepinus* fed 1% *A. vera* had a significantly enhanced growths stating that, lower FCR as well as higher Protein Efficiency Ratio (PER) were also observed for fish with 1%, concluding that, *A. vera* polysaccharide extracts suitable to enhance the growth of *C. gariepinus* was between 1.76% and 1.79%.

2.9 **Effects of plant extracts on blood parameters**

Blood parameters are indicators of health and give information on the status of almost every part and organ in the body, haematological changes are characteristically used to evaluate physiological, environmental and pathological indicators of fishes and other

animals, this is supported by the several reports of the many contributions of haematological studies on successful aquaculture (Akinrotimi *et al.*, 2012; Gabriel *et al.*, 2011; Satheeshkumar *et al.*, 2011). Many researches have been conducted to support the safe use of phytochemicals on fish relating to ascertaining the health conditions of these fish species. 34 – 37

Extracts of some plants; *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera* and *Zingiber officinale* were studied to assess the effects on the blood parameters of *Oreochromis mossambicus* where acetone extracts of *C. dactylon*, *A. marmelos*, *W. somnifera* and *Z. officinale* were supplemented in the diets of *O. mossambicus* and fed for 45days at 5% body weight. Packed cell volume of this fish was higher (34.16% – 37.95%) than those of the control fish while leucorit value were enhanced in treated fish. Plasma biochemistry showed that plasma parameters such as albumin, cholesterol, globulin, glucose protein and triglyceride levels were significantly higher in treated *O. massambicus* than those of control group (Immanuel *et al.* 2009). Effects of *Melissa officinalis*and *Aloe vera* on *O. mykiss* were investigated by Farahi *et al.* (2012) by feeding *O. mykiss* diets supplemented with ground *M. officinalis* at 20 g/kg and *A. vera* at 10 g/kg and reported that, compared to fish fed 'unsupplemented' diets, fish fed supplemented diets had significantly enhanced white blood cell counts and haematocrit levels however, there was no significant change in the red blood cells and haemoglobin levels.

Similarly, the effect of dietary essential oil of *Citrus sinensis* on the blood parameters of *O. mossambicus* was also studied by Acar *et al.* (2015) where fish were fed with diets supplemented with *C. sinensis* oil at 0.1%, 0.3%, and 0.5% for 90 days. Haemoglobin, haematocrit levels, erythrocyte indices, serum albumin, cholesterol, globulin, glucose total protein and triglyceride were all significantly higher in treated fish than the control group and concluded that *C. sinensis* oil can increase immunity in *O. mossambicus*.Comparing fish fed *B. alba* and 17α-Methyltestosterone, Chakraborty (2017) reported that compared to *O. niloticuss* fed *B. alba* and*T. terrestris* supplemented diets, those fed with 17α-Methyltestosterone had significantly reduced eosinophils, lymphocytes, haemoglobin concentration, haematocrit level, mean corpuscular hemoglobin concentration, monocytes, neutrophils, total Red Blood Cell and total White

Blood Cell. This author further reported that, both plant extracts enhanced the hematological parameters of *O. niloticuss*concluding that, the enhanced haematological parameters of the extracted treated fish proved adaptogenic activity of the extracts.Manaf *et al.* (2016) treated hybrid red tilapia with herbal mixtures of *Vitex trifolia, Strobilanthes crispus* and *Aloe vera* for 60 days to evaluate the haematological parameters of the fish. They observed that, haemoglobin, Red Blood Cell and White Blood Cell showed higher significant difference in all treated fish and the biochemical parameters such as Alanine transaminase, Alkaline phosphatase and Aspartate transaminase of the fish were influenced by the herbal mixture. The authors reported that, haematocrit, haemoglobin, red blood cell counts, plasma globulin and total plasma protein levels all increased with increasing dietary herbal levels compared to those of the control diet fed fish concluding that, these herbal mixtures can stimulate the immune system of hybrid red tilapia. Similarly, Kareem *et al.* (2016) assessed the of the actions of four comestible crude plant extracts on the blood parameters of Nile tilapia and reported that, the effects of dietary extracts of *Azadirachta indica*, *Carica papaya*, *Cinnamomum camphora* and *Euphorbia hirta* on blood parameters and biochemistry of *O. niloticuss* after feeding them diets with these extracts at 2 g/kg for 90 days were not affected except plasma AST which was slightly lower in fish given feed with extracts in contrast with those given feed that served as control diets with no extracts.

The effects of *Phoenix dactylifera* seed extract on haematology and immunophysiological parameters of *Cyprinus carpio*were evaluated by Mohammadi *et al.* (2018) which resulted in lower haematological parameters in fish fed *P. dactylifera* seed extracts above 0.5%, however, those fed 0.5% had similar leucocyte, neutrophil, lymphocyte and monocyte counts as the control fish. They further reported that, albumin and total protein were higher in all treated fish than control group while alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, cholesterol, and glucose levels were lower level in fish fed with 0.5% than in all other treatments. In like manner, Kamali-Sanzigh *et al.* (2018) substituted plant sources in *C. carpio* diets with *P. dactylifera* waste meal by 5%, 10% and 15% and fed them for 12 weeks to determine the effect on the haematology and biochemical parameters. The authors reported that, replacement of plant sources by *P. dactylifera* Waste Meal in diets of *C. carpio* did not affect Mean Corpuscular Volume,

Mean Corpuscular Haemogblobin and all biochemical parameters however, there was significantly Haemoglobin, reduced Red Blood Cell and neutrophil compared to control groups. *Clarias gariepinus* fed diet supplemented with 4.0%/kg *A. vera* showed improved haematological indices compared to those feed control diets in an experiment where *C. gariepinus* were administered with diets enhanced with varying inclusion quantity of *A. vera* po1ysaccharide ranging from 0.5%/kg to 4%/kg for 8 weeks (Gabriel *et al.*, 2019).

2.10 **The use of phytochemicals as fish sex reversal agents**

Phytoestrogens and phytoandrogens with similar structural composition as many steroidal hormones have been reportedly found in several medicinal plants bringing about a number of researches and studies to investigate the potentials of some plants extracts as masculinizing or feminizing agents in fish.

Mangosteen leaf extracts were used to induce sex reversal in *Oreochromis niloticuss* fry after yolk absorption when they were treated in water containing mangosteen leaf extracts in concentrations ranging from 0 g/L to 5 g/L and recorded 73% males in fry fed 2g/L of mangosteen leaf extracts (Khakong *et al.*, 2011). Stadtlander *et al.* (2012) studied the gonad histology and sex ratio of *O. niloticuss* fed feed sprayed with saponins extracted from fenugreek plant *Trigonella foenum-graecum* and two commercial saponins and recorded male percentage ranging from 46% to 57% and reported no significant difference across the treatments. Similarly, Ghosal and Chakraborty (2014a) investigated the potential effects of aqueous *Basella alba* leaf extract to masculinize *O. niloticuss* by immersion technique for 30 days, producing the highest male percentage in fish at 1.0 g/L concentration although, the authors recorded intersex with both male and female gonadal tissues in all treated fish. The efficacy of crude extract of *Butea superba* to produce all male tilapia was tested by Kiriyakit (2014) by feeding three day old *O. niloticuss* with feed supplemented with six concentrations of *B. superba* crude extract ranging from 0 mg/kg to 200 mg/kg in multiples of 40mg and recorded an all-male population (100%) in fish fed with 200 mg/kg which were significantly higher than those fed with other levels. Omar *et al.* (2014) estimated the end result of dietary phytochemicals on the sex reversal of *O. niloticuss* by feeding the fish diets containing containing ginseng extract at 0.2 g/kg

and 0.4 g/kg and date palm extract at 3 g/kg and 6 g/kg for 28 days. The authors recorded the highest male percentage (92.30%) in fish fed MT diets and 56.67% in fish fed with diets containing 6 g/kg date palm extract.

Furthermore, *Mucuna pruriens* seeds aqueous extract was used as dietary and immersion treatments for *O. niloticuss* at concentrations between 2.0 g/kg to 5.0 g/kg and 0.00 g/L to 0.05 g/L respectively for 30 days to produce monosex population, results showed that the highest levels in both treatments produced the highest male percentage of 73.33% and 74.67% respectively, however, survival were lower at these levels (Mukherjee *et al.,* 2015b). Similarly, Ghosal *et al.*, (2016; 2015) assessed the efficacy of *B. alba* leaves extracted with different solvents as well as compared dietary treatment and immersion of *O. niloticuss* in monosex production. The authors reported that extraction with ethanol produced the highest male percentage at 74.4% noting that, the highest concentration produced the most males. They also asserted that, both dietary and immersion produced the highest males of 70.3% and 71.9% at 10 g/kg and 0.1 g/L respectively observing also that, survival were highest at these levels.

In the same trend, Gabriel *et al.* (2017), evaluated the sex ratio of sexually undifferentiated genetically improved farmed *O. niloticuss* administered dietary *Aloe vera* powder for 30 days and reported that, male percentage increase was directly proportional to *A. vera* inclusion level with fish fed 4.0 g/kg producing highest males at 67.62% however, survival presented as inversely proportional with the least survival in 4.0g/kg treatment. Similarly, Ramírez *et al.* (2017) evaluated the gonadal maturation of *O. niloticuss* after feeding the fish extracts of *Passiflora incarnate* for 90 days while Nian *et al.* (2017) used pollens of *Pinus tabulaeformis*as 17-α-methyltestosterone replacement in sex reversing *O. niloticuss* larvae. They incorporated the pollen powder into a 45% crude protein diet at 0.00 g/kg, 0.08 g/kg, 0.16 g/kg, 0.32 g/kg and 0.64 g/kg concentrations and fed the fish *ad libitum* for 8 weeks and recorded as high as 89.1% male in 0.32 g/kg treatments which were not significantly different from those fed 17-α-methyltestosterone. Likewise, Mukherjee *et al.* (2018) administered orally, extracts of *Mucuna pruriens* seeds and *Asparagus racemosus* roots to *O. niloticuss* for 30 days and observed that, fish fed these extracts were significantly higher than the control group

achieving as high as 93.79% and 92.24% males in fish fed with *M. pruriens* seed and *A. racemosus* root extracts respectively.

2.11 **Chemical composition of *T. terrestris* extracted with different solvents**

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 will be 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. 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). Some of the chemical structures of the phytochemicals found in *T. terrestris* are shown in figure 2.1 to figure 2.3. Preliminary phytochemical assessment of *T. terrestris* in Northern Nigeria by Usman *et al.*, (2007) revealed the presence of alkaloids, flavonoids, glycosides, saponins, and tannins. This plant is extremely rich in substances having potential biological significance such as saponins, flavonoids, alkaloids (Gauthaman and Ganesan, 2008), glycosides, phytosteroids (Şahin and Duru, 2010) and other constituents. *Tribulus terrestris* was reported to be a rich source of furostanol and spirostanol saponins and flavonoids by Farooq *et al.* (2012). The phytochemical investigation of aqueous and methanol solutions of *T. terrestris* fruit and leaves by Sharma *et al.* (2013) showed that *T. terrestris* fruit and leaves had alkaloids, carbohydrates, flavonoids, saponins and tannins while Mathur and Sundaramoorthy (2013) reported that *T. terrestris* fruits from different sites with varying growth conditions indicated the presence of alkaloids, proteins, steroidal saponins and soluble and insoluble sugars. The aqueous, ethanol and benzene extracts of *T. terrestris* flowers were reported to contain alkaloid, tannins, saponins, carbohydrates, flavonoid, terpenoids, steroids, anthraquinone, glycosides, and sterols (Sujatha and Prakash, 2013).

Other phytochemicals from ethanol extract of *T. terrestris* reported by Gincy *et al.* (2014) are; flavonoids, saponins, tannins and terpenoids. Additionally, Semerdjieva and Zheljazkov (2019) stated the occurrence of alkaloids, cinnamic acid amides and lignan amides in *T. terrestris*. It contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, etc. (Akram *et al.*, 2011; Dhas *et al.*, 2015).

Shahid *et al.* (2015) further revealed that alkaloids, flavonoids, glycosides, phytosterols and saponins are the biologically active constituents of *T. terrestris*. Vasait, (2017) analyzed the crude aqueous, acetone and methanol extracts of *T. terrestris* leaves and fruits and reported that all extracts revealed the presence of amino acids, carbohydrates, glycosides, phenols, proteins, saponins, tannins, terpenoids and important secondary compounds. Dwivedi and Sengar, (2018) performed phytochemical analysis on fruits, leaves and roots of *T. terrestris* using maceration method of extraction and chloroform, ethanol and water petroleum ether as solvents, the result of this study revealed the presence of alkaloids, flavonoids, saponins, steroids, glycosides and phenolic compounds in the plant concluding that the ethanol and water extracts of leaves and fruits of *T. terrestris* were rich in flavonoids, glycosides, phenolic compounds and saponins. Similarly, Ammar *et al.* (2018) reported that the dried aerial parts of *T. terrestris* using Soxhlet extraction with ether, petroleum ether, methanol and 50% aqueous methanol as solvents contained sterols or terpenes, flavonoids, saponins, alkaloids and nitrogenous compounds.

Tribulus terrestris is an industrial source for production of medicinal preparations based on its saponin fraction being one ofthe richest producers of saponins (Chakraborty, 2017; Ivanova *et al.*, 2009). *Tribulus terrestris* is mainly known for its effectiveness in libido disorders, impotence and infertility. Data have also been published on its cardiovascular, cytotoxic and antimicrobial activities. The steroidal saponins have been considered to be the main active principles of these plants, responsible for their numerous healing applications (Ivanova *et al.*, 2009). It is believed that *T. terrestris* affects androgen metabolism, significantly increasing testosterone or testosterone precursor levels (Yeganeh *et al.*, 2017).

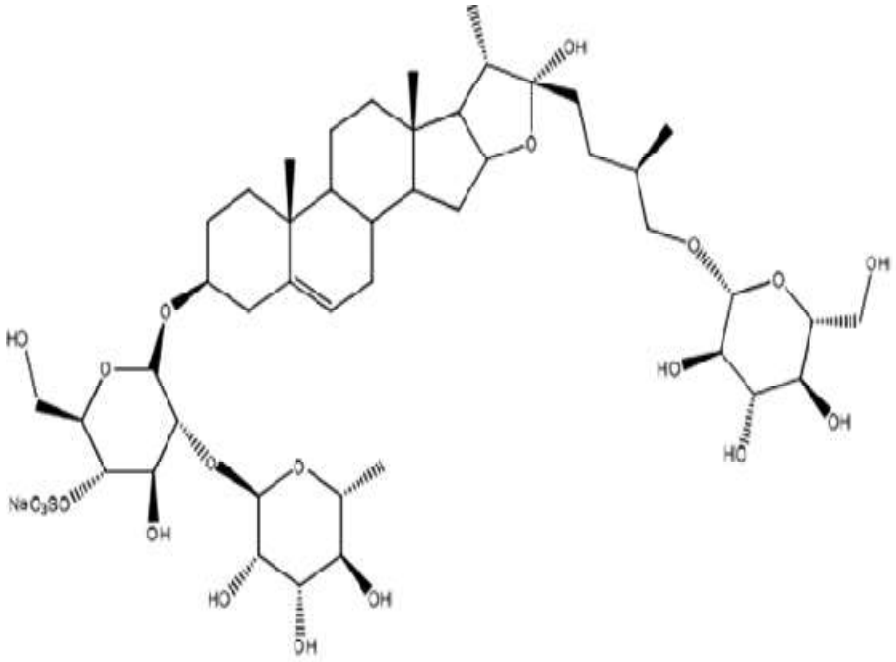

Fig. 2.1: Chemical Structure of the Steriodal Saponin Prototribestin found in *T. terrestris* (Adapted from Semerdjieva and Zheljazkov, 2019)

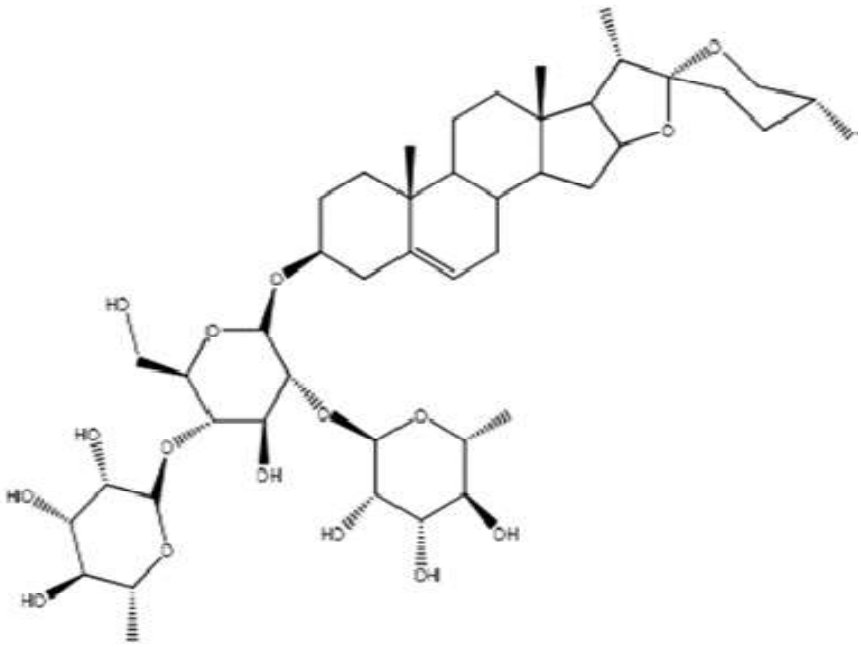

Fig. 2.2: Chemical Structure of the Steriodal Saponin Dioscin found in *T. terrestris* (Adopted from Semerdjieva and Zheljazkov, 2019)

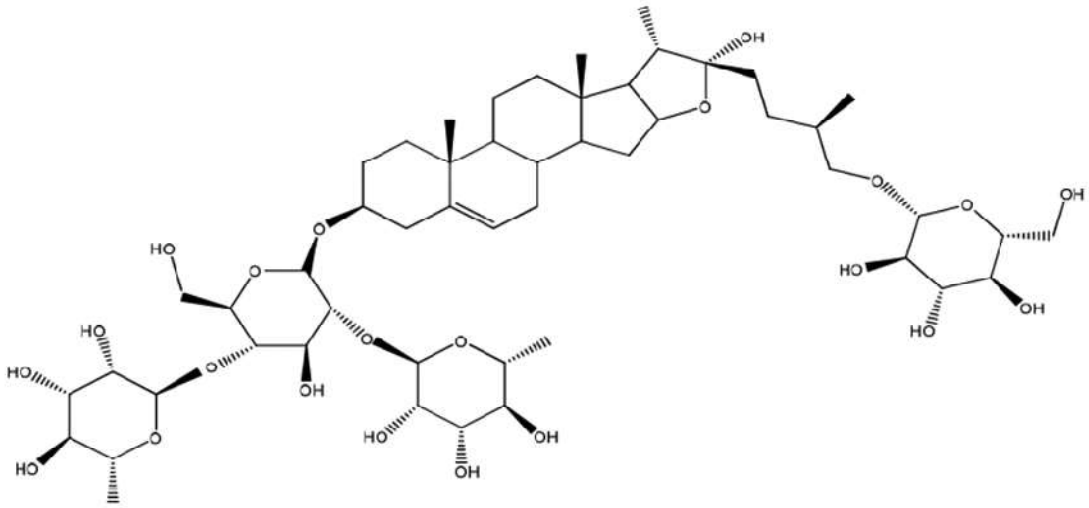

Fig. 2.3: Chemical Structure of the Steriodal Saponin Protodioscin found in *T. terrestris* (Adopted from Semerdjieva and Zheljazkov, 2019)

The basic medicinal properties of *T. terrestris* are defined by the presence of steroidal saponins having a furostanol or spirostanol nucleus, glycosylated in 3 or 26 positions with linear and branched glycosidic units, these are explicit phytochemicals in *T. terrestris* and have influenced many studies on this steroidal saponins (Semerdjieva and Zheljazkov, 2019). Although the furostanol saponins (Turan, 2017) are the main components of *T. terrestris* of Bulgarian origin, only methylprotodioscin, protodioscin, methylprototribestin and prototribestin have been isolated and identified so far (Chhatre *et al.*, 2014). Other constituents like amino acids, fatty acids, polysaccharides, and potassium salts have been isolated from *T. terrestris* (Umadevi and Srinathrao, 2017). Anthraquinones in addition to other already mentioned constituents were found in the methanol, chloroform and petroleum extracts by El-Shaibany *et al.* (2015).

Tribulus terrestris contains a number of different substances known as steroidal saponins. Protodioscin, the most dominant saponin in *T. terrestris*, is thought to be main substance responsible for increasing testosterone production (Janalizadeh *et al.*, 2019; 2018). Protodioscin, the main active components of *T. terrestris* is a saponins of furostanol type and has also been reported to increase levels of non-hormonal phytodehydroepiandrosterone (DHEA), dihydrotestosterone and dehydroepiandrosterone sulphate (Janalizadeh *et al.*, 2019; 2018).

As a source of nutrient, *T. terrestris* leaves have been reported to contain 79.09% moisture content, 7.22% protein, 0.08% phosphorous, 1.55% calcium, 9.22 mg iron/100g of the leaves and 41.5 mg vitamin C.

2.12 ***Tribulus terrestris*** **as a medicinal plant**

*Tribulus terrestris*is commonly known as Gokshur (Sanskrit); puncture vine, land (or small) caltrops, Goat head, Devil's thorn and yellow vine (English); Gokharu (Hindi); Bethagokhura or Nanagokharu (Gujarathi); Nerinjil (Tamil) and Khar-e-khusak khurd (Urdu); croix de Malte (French); Abrolhos (Portuguese); demir dikeni (Turkey) and in Nigeria, Dareisa (Arabic-Shuwa); tsaiji (Fulfulde); Hana taakama and Tsaida

(Hausa); Kaije (Kanuri); tedo (Koma in Adamawa State) and da ogun daguro (Yoruba) (Chhatre *et al.*, 2014; Muanya, 2015).

Taxonomical classification of *T. terrestris* according to Chhatre *et al.*, (2014)

Kingdom: Plantae

Phylum: Phanerogams

Subphylum: Angiospermae

Class: Dicotyledonae

Subclass: Polypetalae

Series: Disciflorae

Order: Giraniales

Family: Zygophyllaceae

Genus: *Tribulus*

Species: *terrestris* Linn

2.12.1 **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 is a mat forming prostrate plant that reproduces by seed. It 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 in a few weeks; flowers may be produced within 3 weeks, fruits within 6 weeks (Moradikor *et al.*, 2013). *Tribulus terrestris* has tap root system with slender, branched and woody, deep taproot with a network of fibrous roots which has been reported 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 flower, it continues through out. 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). Each fruit section has two sharp divergent spines and several other spines enabling the *T. terrestris* fruits to easily attach to animals and humans (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). Most newly matured seeds are dormant and require an after ripening period of approximately one year, water absorption of dormant sees differed from non-dormant seeds. *Tribulus terrestris* germination has been reported to be inhibited by low temperatures, low light intensities, and wet soil. The optimum temperature range for this plant to germinate is between 27 ^{0}C to 35 ^{0}C. *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, maximum germination occurred after a series of heavy rains, facilitating a 35% germination rate, with continued germination of seeds lasting for another four months.

2.12.2 **Therapeutic applications of *T. terrestris***

Tribulus terrestris is a widespread medical plant used by physical activity practitioners mainly due to commercial claims (Pokrywka *et al.*, 2014). The whole of the aerial parts of *T. terrestris*; leaves, fruits, flowers and stem have been reported to have medicinal properties and have been used for such purposes and these have no taste or smell (Semerdjieva and Zheljazkov, 2019). Early studies showing beneficial effects of the use of *T. terrestris* were performed by a pharmaceutical company in Bulgaria. Preliminary results have shown that the supplementation raised plasma TST and, consequently, would promote gains in lean mass and strength. Authors proposed that weightlifting athletes would improve performance with *T. terrestris* supplementation and a protein-rich diet, suggesting an important effect caused by the presence of naturally occurring saponins in the plant, which are related to traditional mechanisms and/or androgenic pathways such as the release of LH and TST (Pokrywka *et al.*, 2014)

2.12.3 **Toxicity of *Tribulus terrestris***

Many studies have found that numerous plants used as food, spices, or in traditional medicine have endocrine disrupting, carcinogenic, or mutagenic characteristics, despite claims to the contrary (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. 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 420 µg/ml respectively (Divya *et al.*, 2014). In humans, the growth of human prostrate 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; 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 excretion and metabolic degradation of saponins, since large doses may lead to plasmatic accumulation and induction of membrane lysis as well as apoptosis of erythrocytes.

2.12.4 ***T. terrestris*** **extract as a growth promoter in fish**

Tribulus terrestris has been acclaimed by several researchers to support growth and muscle development in both man and animals. 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. The growth promoting effects of *T. terrestris* extract were recorded during immersion experiments on convict cichlid, *Cichlisoma nigrofasciatum* and guppy, *Poecilia reticulata* (Cek *et al.* 2007a and 2007b). These authors reported that, *T. terrestris* extract treated fish exhibited successful growth acceleration and significantly higher growths compared with the control group, these results are in line with the findings of Turan and Cek (2007) who observed the highest body weight gain in *Clarias gariepinus* fed 9 g *T. terrestris* extracts for 30 days.

Kavitha and Subramanian (2011) measured the growth 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 for two months. They reported accelerated growth in terms of total length and body weight in all the treated fish compared with the control group with those fed 30 ppm/L producing the highest growths. Yilmaz *et al* (2013) treated all-female population of *O. 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 *O. niloticuss* fed different levels between 200 mg/kg to 600 mg/kg of *T. terrestris* extracts. Similarly, Omar *et al.* (2014) fed *O. niloticuss* diets with 0.6 g/kg and 1.2 g/kg *T. terrestris* extracts to determine the effect of this dietary addition of *T. terrestris* extracts on the growth performance of the fish after 28 days and 84 days. At the end of 28 days, fish fed with 1.2 g/kg *T. terrestris* had the highest fina1 b0dy weight and specific growth rate while at th/e of 84 days, fish fed MT diets had the highest growth followed by those fed diets with 1.2 g/kg with no significant differences between them. They concluded that, growth of *O. niloticuss* 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 1 g/kg (123.83 g and 1.76%) and 2g/kg (121.13 g 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 10 mg/L *T. terrestris* and 200 mg/L *U. dioica* was reported to improve growth indices of *C. nigrofaciatum*, this was against the backdrop that growth of *C. nigrofaciatum* declined when fed only *U. dioica* extracts(Babahajiani *et al.*, 2018).

2.13 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 it is used as an aphrodisiac. It is also used as a diuretic and nervine tonic, where as in Unani medicine to inhibit the formation of kidney stone (Aggarwal *et al.,* 2010). *Tribulus terrestris* reportedly increased sperm count as well as motility levels when it was taken for 30 days

according to Akram *et al.* (2011) which led to the conclusion that *T. terrestris* is a good supplement for men and women to increase their sex drive. 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.*, 2015a; Ghosal and Chakraborty, 2014a; 2014b; Hu *et al.*, 2014; Fe1icitta *et al.*, 2013; Bai *et al.*, 2012). Some phytochemicals in plants known as phytoestrogen have been reported by Das *et al.* (2012) to emulate 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 common therapeutic assertions of *T. terrestris* use is its potential to improve libido and erectile dysfunction, however, Santos *et al.* (2014) reported studies with adult men and women where there were no significant results in the male individuals but a considerable libido improvement in the female individuals. An indication of *T. terrestris* stimulating an increase 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 deficiency, the authors observed a significant difference in the testosterone leve1s 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 fed 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 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. *Tribulus terrestris* was used to improve sex hormones in opium addicted rats as reported by Ghosian *et al.* (2013) while 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 198 mg/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 (FCR) 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 humans, it has been used to treat erectile dysfunction and to increase testosterone levels while improving athletic performance (Singh *et al.*, 2012; Porkrykwa *et al.*, 2014). Several other researches on the effects 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 ar0matase inhibit0rs and nuc1ear estr0gen receptor antagonists in g0nad germ ce11s (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.*, 2015b). *Tribulus terrestris* is ingested by peop1e, allegedly for musc1e bui1ding and has dem0nstrated hepat0-pr0tective and antiοxidant activities in *O. mossambicus* (Kavitha *et al.*, 2011). Current research investigated the 0ptimum dosage of *T. terrestris*, and its effects 0n the growth perf0rmance, haematologica1 and biochemica1parameters of *O. niloticuss*.

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-Si1va *et al.*, 2013; Baroi1ler *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 3 mg and 6 mg 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. Prior to the current study, Omitoyin *et al.* (2013) achieved 83.7% masculinization of *O. niloticuss*with commercial *T. terrestris* extract at 2.0 g/kg.

Similarly, Ghosal and Chakraborty (2014b) recorded 81.4% males in treating *O. niloticuss*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. niloticuss* 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 fed 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; 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 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.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Phytochemical Composition of *T. terrestris* Extracted with Different Solvents.

Different solvents (ethanol, ethyl acetate and water) with varying polarities were used to process experimental plant *T. terrestris* whole plant (leaves, stem, seeds and root) to ascertain the various chemical constituents in the whole plant.

3.1.1 Experimental site

The extraction and phytochemical screening of *T. terrestris* was carried out in Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Chemistry, University of Ibadan, Ibadan, Nigeria. The University of Ibadan is situated in the Western region of Nigeria on latitude 7°26'57.27" and Longitude 3°53'8.93".

3.1.2 Sample collection

*Tribulus terrestris*were collected from Abubakar Tafawa Balewa University (ATBU) Bauchi and its environs, Bauchi, Nigeria. Bauchi is located in Northwestern part of Nigeria. The sample was identified and authenticated at the University of Ibadan Herbarium, Department of Botany. A voucher sample with code UIH-22848 was deposited at the Herbarium.

3.1.3 Sample preparation

Whole *T. terrestris* plant (leaves, stem, seeds and roots) were cleanedto remove soil and debris, then evenly spread on trays, covered with cotton sheets, this is to prevent dust and insects getting in contact with the plant and left in a room (27 ^{0}C) with cross ventilation for seven days to dry. This plant samples were turned on the tray daily to ensure uniform

dryness. The dried *T. terrestris* samples were weighed and pulverized in a clean dry miller according to the methods of Kiran *et al.* (2011).

3.1.4 **Extraction procedure**

Five solvents with increasing dielectric constant (polarity) were selected for the purpose of extraction; ethyl acetate (0.228); ethanol (0.654); methanol (0.762) and water (1.000) (Murov, 2018). This solvents used for the extraction of *T. terrestris* were chosen based on the closeness of their polarity to the polarity of phytochemicals in *T. terrestris* (Ganzera *et al.*, 2001) since a solvent of similar polarity to the phytochemicals will properly dissolve the compound (Altemimi *et al.*, 2017). The extraction procedures for this study were by means of maceration and were carried out according to methods adapted from Singh (2008); Azwanida (2015).

The pulverized samples were weighed accurately (100g) and placed in air tight containers of five litres (5 L) each infused with aqueous ethanol (95%), ethyl acetate, distilled water at room temperature (27 ^{0}C) and at 100 ^{0}C until fully immersed and was left for 72 hours with constant shaking. After 72 hours, the mixtures were drained and the drained solvents were filtred using a Whatman filtar paper (125 mm) and moved to a rotary evaporator at 60 ^{0}C to concentrate the extracts and afterwards, air dried at room temperature and stored in refrigerated air tight glass jars (Al-Bayati and Al-Mola, 2008; Cathrine and Nagarajan, 2011).

3.1.5 **Phytochemical screening**

The phytochemical screening was carried out at the Department of Pharmacognosy, University of Ibadan. The extracts (ethanol, ethyl acetate and aqueous (at 27 ^{0}C) and (at 100 ^{0}C) were tested for the presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins, anthraquinones, terpenoids, cardiac glycosides and steroid using the standard procedures according to Sofowora (1993); Trease and Evans (2002); Tiwari *et al.* (2011); Obadoni and Ochuko (2002).

3.1.5.1 **Test for alkaloids**

Exactly 100 mg of each extract was dissolved in 5 mL of methanol and then 2 mL of this was mixed with 5 mL of dilute hydrochloric acid. Accurately, 1 mL of this mixture was placed separately into three individual test tubes. In one test tube, 3 drops of Mayer's reagent were added and to the second test tube, Dragendorff's reagent were added while to the third test tube, few drops of Wagner's reagent prepared by adding 1.25 g of iodine and 2 g of potassium iodide to 100 mL of distilled water. The appearance of yellow colour in the first tube, orange-red colour in the second and reddish brown colour in the third indicated the presence of alkaloids in the extracts (Sofowora, 1993; Jindal *et al.*, 2013).

3.1.5.2 **Test for flavonoids**

In five different test tubes, precisely 0.5g of each extract was placed and 2ml of sodium hydroxide solution was added to each test tube, the mixture turned deep yellow which eventually became colourless on dropwise addition of dilute hydrochloric acid. This colour change indicated the presence of flavonoids (Trease and Evans, 2002). A confirmatory test for flavonoid were conducted using the methods of Edeoga *et al.* (2005). Exactly, 0.5g of each extract was heated with 10 mL of ethyl acetate in a 50mL over a steam bath for three minutes, the mixture was filtered and 4ml of the filtrate shaken with 1ml of dilute ammonia solution. A yellow colour confirmed the presence of flavonoids in the extracts (Edeoga *et al.*, 2005).

5.1.5.3 **Test for saponins**

In individual test tubes, 1 g of each extract was dissolved in 10 mL of distilled water in a water bath for 10 minutes, the mixture was filtered while hot and allowed to cool. Additional 3 mL of distilled water was added to the filtrated and shaken vigorously then allowed to stand for 30 minutes. Formation of foam and honey comb froth after standing indicated the presence of saponins in the extracts (Sofowora, 1993).

5.1.5.4 **Test for tannins**

Precisely, 0.5 g of each extract in 10 mL of water was boi1ed in a test-tube for five minutes in water bath and then filtered while hot and allowed to cool down. In a 10 mL beaker, 1 mL of the cooled filtrate was diluted to 5ml with distilled water and a 3 drops of 10% ferric chloride was added. A distinct lack of blue black precipitate in the mixture indicated the absence of tannins in the extracts (Trease and Evans, 2002).

5.1.5.5 **Test for anthraquinones**

Accurately, 1.0 g of each extract were placed in a dry test tube and 20 mL of chloroform was added. These were heated over a steam bath for 5 minutes and filtered. To each filtrate, 5 mL of 10% ammonia solution was added and shaken. A brink pinkish colour was observed in the upper layer which indicated the presence of anthraquinones in the extracts (Sofowora, 1993).

5.1.5.6 **Test for terpenoids (Salkowski test)**

In a 10 mL beaker, 2ml chloroform and 3ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was mixed and exactly 0.5g of *T. terrestris* extract was added. This formed a layer and a dark reddish colour at the interface which indicated the presence of terpenoids (Sheel *et al.*, 2014).

3.1.5.7 **Test for Cardiac Glycosides**

Exactly 0.5g of each extract were dissolved in 10 mL of distilled water in a 50 mL beaker. A solution of 2 mL glacial acetic acid with 1 drop of Iron (III) chloride was added to the dissolved extract. This mixture was carefully added to 1 mL of concentrated tetraoxosulphate (VI) acid in a test tube. This was carried out in such a way that the mixture was placed on the concentrated H_2SO_4**.** The absence of a brown ring between

these layers indicated the absence of cardiac glycosides in the extracts (Edeoga *et al.*, 2005).

3.1.5.8 **Test for steroid**

Exactly 0.5g of each extract was placed in a test tube and dissolved in 10 mL of chloroform and filtered in equal proportion into two other test tubes. To the first portion, 2mL of acetic anhydride was added, accompanied by the addition of 2 mL concentrated tetraoxosulphate (VI) acid in a dropwise manner along the wall of the test tube to form a layer beneath the initial mixture. The appearance of a greenish colour at the point indicated the presence of sterols. As a confirmatory test, to the second portion in a test tube, 2 mL concentrated tetraoxosulphate (VI) acid slowly so that the acid formed a layer on the mixture in the test tube. At the interface, a deep reddish colour confirmed the presence of steroid (Sofowora, 1993).

3.1.6 **Quantitative analysis of the phytochemical constituents**

3.1.6.1 **Determination of Alkaloid**

Exactly 5 g of*T. terrestris* whole plant is measured in to a 500 mL beaker and 200 mL of 10% acetic acid in ethanol is added, covered with a foil and allowed to stand for 4 hours. The mixture then filtered and the extract is allowed to become concentrated in a water bath till it reaches 40mL. While precisely, 1g of each extract is dissolved in 50 mL of 10% acetic acid in ethanol, covered and allowed to stand for 4 hours as well. The concentrated extracts were made alkaline by addition of concentrated ammonium hydroxide is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which is then dried and weighed. Percentage alkaloid is expressed by equation 3.1;

$$\text{Alkaloids (\%)} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} X\ 100 \quad \text{----------} \quad 3.1$$

(Harbone 1973)

3.1.6.2 **Determination of flavonoid**

An accurate measurement of 100 mL of 80% aqueous methanol was added to precisely 5 g of *T. terrestris* whole plant sample in a 250 mL beaker, covered and allowed to stand for 24hours at room temperature (approximately, 25^0C). Equal amount of each extract was also measured in to individual 250mL beakers with the same volume of 80% aqueous methanol added to it. The individual solutions from whole plant and extract were filtered through Whatman filter paper (125mm), the individual filtrates were then transferred into crucibles on a water bath and were evaporated into dryness until a uniform weight was obtained. Flavonoid contents in the whole plants and extracts were calculated as a percentage using the formula in equation 3.2;

$$\text{Flavonoid (\%)} = \frac{\text{Weight of flavonoid}}{\text{Weight of sample}} X\ 100 \text{ ---------- } 3.2$$

(Bohm and Kocipai-Abyazan 1994; Ejikeme *et al.*, 2014).

3.1.6.3 **Determination of Saponin**

For the whole plant, 5g of pulverized sample was accurately measured and placed in a conical flask and 100 mLof 20% aqueous ethanol was added, this was heated over a hot bath for 4 hours with continuous stirring at about 55^0C. The plant and ethanol mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. While 5g of each extract was also dissolved in 100 mL of 20% aqueous ethanol in a 200 mL beaker. Both whole plant extracts and individual solvent extracts were reduced to 40 mL over water bath at 90^0C and were transferred to a 250 mL separating funnel where 20 mL of diethyl ether was added to each and shaken vigorously for purification. The aqueous layer was recovered and the ether layer discarded for each extract. This purification process was repeated with 60ml of n-butanol was added and washed twice with 10ml of 5% aqueous sodium chloride. The sodium chloride layer was discarded and the remaining solution was heated in a water bath for 30 minutes to evapourate. After evapouration, the sample was dried in an oven to a constant weight and the saponin content calculated as a percentage using the formula in equation 3.3;

$$\text{Saponin (\%)} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} X\ 100 \qquad 3.3$$

(Obadoni and Ochuko, 2002)

3.1.6.4 **Determination of steroid**

Accurately, 0.5g of each extract was weighed into a test tube and a 50mL of distilled water was added and shaken vigorously for some time. From these reconstituted extracts, exactly, 2mL was placed in a test tube and washed with 3mL of 0.1 M of sodium hydroxide. This preceded the addition of 2 mL and 3 mL of chloroform as well as refrigerated acetic anhydride respectively to the alkalinized solution and a dropwise addition of 1 mL of concentrated tetraoxosulphate (VI) acid to the mixture. The absorbance of the mixtures and blank were measured spectrophotometrically at 420 nm wavelength (Mbaebie *et al.*, 2012).

The addition of 2 mL chloroform and 3 mL refrigerated acetic anhydride preceded a dropwise addition of 1 mL of concentrated tetraoxosulphate (VI) acid to the alkalinized solution. The absorbance of the mixtures and blank were measured spectrophotometrically at 420 nm wavelength (Mbaebie *et al.*, 2012).

3.1.7 **Selection criteria for experimental extracts**

After quantitative analysis of the different extracts, extracts that had the highest saponin and steroid contents were selected and used for the experiment.

3.1.8 **Proximate analysis of *T. terrestris* extracts**

The proximate composition of *T. terrestris* whole plant and extracts was done at the Central Nutrition Laboratory, Department of Animal Science, University of Ibadan. Samples were analyzed in triplicates and followed the procedures of analysis (AOAC, 2005; FAO, 2003; Ilodibia *et al.*, 2014; Kabir *et al.*, 2015).

3.1.8.1 **Moisture determination**

This was done by oven drying. Exactly 5.0 g each sample (*T. terrestris* and extracts) were individually weighed into weighed crucibles (W_1). The sample was dried in the oven at 100 ^{0}C for 24 hours until a constant weight was obtained. Thereafter, the samples were transferred to a dessicator and cooled for 30 minutes. The cooled samples were then weighed. The weight of moisture lost was calculated and expressed as a percentage of the weight of sample analyzed. . It was calculated as a percentage as in equation 3.4:

$$Moisture\ (\%) = \frac{W1 - W2}{\text{Weight of sample}}\ x\ 100 \quad \text{---------- } 3.4$$

(AOAC, 2005)

3.1.8.2 **Ash content determination**

This was done by furnace incineration gravimetric method. Clean empty crucibles were held over a Busen burner for 5 minutes each and cooled in a desiccator before they were weighed (W_1). Precisely, 1 g of each extract as well as whole plant were weighed in each weighed crucible (W_2) and heated over busen burner until charred, then the crucibles were transferred to a muffle furnace at 550^{0}C for 4 hours till they were completely ashed. The crucibles were cooled completely and weighed again (W_3). Percentage ash content for each of the extract and whole plants were calculated using following formula in equation 3.5:

$$Ash\ content\ (\%) = \frac{\text{Difference in weight of ash } (W3 - W1)}{\text{Weight of sample } (W2 - W1)}\ x\ 100 \quad \text{---------- } 3.5$$

(AOAC, 2005)

3.1.8.3 **Crude fat determination**

This was determined by ether extract method using Soxhlet apparatus where accurately, 1 g of individual extracts were wrapped in a Whatman filter papers were placed in fat-free thimbles and then added to the extraction tube. The thimbles

were put in a soxlet reflux flask and mounted into a weighted extraction flask containing 200 mL of diethyl ether. The solvent was heated, boiled, vaporized and condensed into extraction cups for 1 hour. After extraction, the samples were transferred to a water bath where the solvent was removed via evapouration, the cups with contents were cooled and weighed. The percentage crude fat was calculated using the formula in equation 3.6:

$$Crude\ fat\ (\%) = \frac{\text{Weight of extraction cup and oil} - \text{Weight of empty cup}}{\text{Weight of sample}}\ x\ 100 \text{ --- } 3.6$$

(AOAC, 2005)

3.1.8.4 **Crude fibre determination**

An accurate measurement of 1.0 g of each extract and plant sample were placed in a 500 mL beaker and boiled in 150 mL of 1.25% tetraoxosulphate (VI) acid solution for 30 minutes. The boiled samples were washed in water using saran cloth to collect the particles, which were returned to the beakers and boiled further for another 30 minutes in 150 mL of 1.25% sodium hydroxide. After cleaning the samples by washing, they were allowed to drain dry before they were transferred to weighed crucibles, then oven-dried at 100^0C to a constant weight before they were transferred to a muffle furnace and burnt to ash. The weight of the fibre in each sample was determined by difference and calculated as a percentage of the weight of sample analyzed with the formula in equation 3.7:

$$Crude\ fibre\ (\%) = \frac{(W2 - W3)}{\text{Weight of sample}}\ x\ 100 \text{------- } 3.7$$

(AOAC, 2005; Ilodibia *et al.*, 2014)

Where;

W_2 = Weight of crucible +sample after washing, bioling and drying

W_3 = Weight of crucible + ash

3.1.8.5 **Crude protein determination**

This was done by kjeldahl method the total nitrogen was determined and multiplied with factor 6.25 to obtain protein content. Exactly 1 g of extracts and plant sample were mixed with 10 mL of concentrated H_2SO_4 in digestion flask and copper (II) sulphate was used as the catalyst to increase digestion. The digested sample was diluted to 100 mL in a 500 mL volumetric flask and used for the analysis. The 10 mL of the digest was mixed with equal volume of 40% NaOH solution and made alkaline, there by producing ammonium sulphate. The released ammonia which was collected in 25mL 2% boric acid solution and titrated against standard HCl until it turned grey. Total protein for each extract was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) using the formula as shown in equation 3.8:

$Crude\ protein\ (\%) = 6.25\ x\ \%N$ ----------*3.8* (Kabir *et al.*, 2015)

3.1.8.6 **Carbohydrate determination**

The total carbohydrate content (%) also referred to as Nitrogen free extract in the samples were calculated by difference method as the subtraction of the sum of the crude protein, total fat, moisture, and ash from the total weight of the sample. Total carbohydrate is calculated using the formula in equation 3.9:

$Total\ Carbohydrate\ (\%) = 100\ -\ ([moisture + protein + fat + ash + fibre]$g in 100g of food) ---------- *3.9*

3.1.8.7 **Total energy value**

The total energy value in the extracts and whole plant of T. terrestris in kcal/100 g was estimated using method described by FAO (2003) as shown in equation 3.10 below:

$Energy\ value\ (Kcal/g)\ =\ ([\%\ crude\ protein\ x\ 4.0]\ +\ [\%\ crude\ fat\ x\ 9.0]\ +\ [\%\ carbohydrate\ x\ 4.0])$ ---------- 3.10 (FAO, 2003)

3.1.9 **Proximate ana1ysis of experimental diets and treated fish**

The proximate composition of experimental fish fed diets containing varying inclusion levels of *T. terrestris* and the diets were carried out as described in 3.1.8.

5.2 **Growth performance of *O. niloticuss*in the laboratory phase**

5.2.1 **Larval production**

Adult *Oreochromis niloticus* broodstocks consisting of twelve males and thirty six females of average weight between 350 g and 500 g were procured from a reputable tilapia farm in Lagos State, Nigeria and transported in 60 L fish transport containers to the Fish farm of the Aquaculture and Fisheries Department, University of Ibadan and stocked separately in 1m x 1m indoor concrete tanks for 14 days to condition them while they were fed with commercial diet (CP 40%) twice daily. After acclimatization, broodstocks were stocked in 1m X 1m hapas rigged in an earthen based concrete tanks at a ratio of 1:3 (male to female). Water depth was maintained at 0.6 m for 14 days while feeding three times daily. The fish were examined eve for fertilized eggs. The fertilized eggs were then collected and incubated in 0.65 m x 0.57 m x 0.3 mrectangular fiber glass incubation troughs in the hatchery for between 15 hours and 18 hours while being aerated via air stones powered by Resun ACO-008 electromagnetic air pump for the eggs to hatch. The day-old fry were separated from other unhatched eggs by siphoning and transferred to 60 L holding troughs before they were distributed into 42 0.55 m x 0.35 m x 0.4 mplastic tanks.

5.2.2 **Feed formulation and preparation**

Based on the recommendations of Mjoun *et al.* (2010) that the crude protein requirement for tilapia fry should be between 30% and 56%, a standard diet of 45% C.P was prepared using the following feed ingredients purchased from a local market and milled; soy bean, maize, fish meal, vitamins premix, di-calcium phosphate, and salt (Table 3.1a and 3.1b). The milled feedstuff was passed through a 200 µm sieve mesh (saran cloth) and a basal

diet was formulated. After the screening and quantitative analysis of phytochemicals contained in *T. terrestris* extract, based on quantity of saponins and steroid contained in each extract, two with the highest of these were chosen for oral administration to *O. niloticuss* larvae. Diets containing 0.0 g, 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g of aqueous (at 27 ^{0}C) and ethanol extracts of *T. terrestris* each to 1 kg of basal diet served as experimental diets according to Omitoyin *et al.* (2013); Ferreira *et al.* (2016). The control diet with 17-α-methyltestosterone (MT) was prepared by adding 50 mg to 1 kg of the basal diet using the evapouration method according to Guerrero (1975).

5.2.3 **Experimental Design and Setup**

The experiment was a 2x7 factorial design. This was made up of two processing methods (extraction) and seven inclusion levels for each. The experiment consisted of six treatments and one control in three replicates for each extract. After three days post-hatching when the yolk had been absorbed, seventy-five fry were randomly distributed into each of 60L capacity plastic tanks with 35L of water. Hatchlings were transferred from hatchery and conditioned for three days until they absorbed their yolks. The experiment was carried out for 42 days which is in line with El-Griesy and El-Gaman (2012).

5.2.4 **Experimental procedures**

Weighed fry (through wet weighing; the fry were placed in a known weighted bowl and known volume of water) were reared for 42 days this is in line with the recommendations of El-Griesy and El-Gaman (2012). Feeding the fry with experimental diets commenced from day four (day one after yolk-sac absorption) and were fed to apparent satiation thrice daily. The tanks were cleaned and settled fish wastes siphoned with half of tank's water each day and fresh aerated water from the overhead tank connected to the laboratory from the storage tank was used to replenish the tank to 35L. Water was changed completely twice a week. Water quality parameters (pH, Temperature, Dissolved Oxygen, Nitrite and Ammonia) were monitored and recorded weekly.

Table 3.1a. Gross composition of feed ingredients for experimental diet

Feedstuffs	% C.P	% inclusion (calculated)	g/kg of diet	% in kg basal diet (analyzed)
Fishmeal	72	25.55	255.5	
Soy bean meal	42	53.90	539	
Maize	10	18.05	180.5	
Dicalciumphosphate (DCP)		1.00	10	
Vitamins premix		1.00	10	
Salt		0.50	5	
Total		100	1000	
Crude protein (%)		45		45.6
Ash (%)		5.9		6.3
Crude fibre (%)		5.5		5.55
Crude fat (%)		3.9		4.1
Moisture (%)				7.26

Table 3.1b. Gross composition of experimental diets containing *T. terrestris* aqueous (at room temperature) and ethanol extracts

Ingredient % (g/100g DM)	Control (MT)	0.0	$TTA_{27}0.5$	$TTA_{27}1.0$	$TTA_{27}1.5$	$TTA_{27}2.0$	$TTA_{27}2.5$	TTE0.5	TTE1.0	TTE1.5	TTE2.0	TTE2.5
Fishmeal	25.55	25.55	25.55	25.55	25.55	25.55	25.55	25.55	25.55	25.55	25.55	25.55
Soy bean meal	53.90	53.90	53.90	53.90	53.90	53.90	53.90	53.90	53.90	53.90	53.90	53.90
Maize	18.15	18.15	18.15	18.15	18.15	18.15	18.15	18.15	18.15	18.15	18.15	18.15
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamins/mineral premix*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100
MT	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T. terrestris	0.00	0.00	0.05	0.10	0.15	0.20	0.25	0.05	0.10	0.15	0.20	0.25
Crude protein (%)[1]	45.6	45.6	45.6	45.6	45.6	45.6	45.6	45.6	45.6	45.6	45.6	45.6

*1kg of Vitamins premix contains in (g/kg premix): Vitamin B1 (Thiamine): 20; Vitamin B2 (Riboflavin): 20; Vitamin B6 (Pyridoxine): 10; Vitamin B5 (Pantothenic acid): 50; Inositol: 100; Vitamin B7 (Biotin): 1; Vitamin B9 (Folic acid): 5; Para aminobenzioc Acid Choline: 500; Vitamin B3 (Niacin/nicotinic acid): 100; Vitamin B12 (Cyanocobalamin): 1; Vitamin A (Retinol) (IU/kg): 5,500; Vitamin E (α-tocopherol) (IU/kg): 50; Vitamin K: 10; Vitamin C (Ascorbic acid): 375; Vitamin D3 (Cholecalciferol) (IU/kg): 2,000; Manganese: 30; Iron: 35; Zinc: 45; Copper: 3; Iodine: 5; Cobalt: 2; Lysine: 85; selenium: 0.15; Antioxidant: 80

[1]laboratory analyzed crude protein

Mortality in each tank of the different treatments were recorded and removed daily and fish were weighed weekly with B5003T electronic weighing balance (minimum level 0.01g – maximum level 500g). At the end of 42 days, fish were weighed for each treatment and the fish were transferred to the Department of Aquaculture and Fisheries fish farm for the pond phase.

Growth parameters and nutrient utilization such as Mean Weight Gain (MWG), Average Daily Growth (ADG), Specific Growth Rate (SGR), as well as Survival rates were monitored and calculated using the following formulas shown in equations 3.11 to 3.14 by Sveier *et al.* (2000).

$$\textbf{Survival Rate}\ (\%) = \frac{\text{Number of fish that survived}}{\text{Total number of fish stocked}}\ x\ 100 \text{----------} \quad 3.11$$

$$\textbf{Weight gain}\ (\textbf{g}) = W_2 - W_1 \quad \text{----------} \quad 3.12$$

$$\textbf{ADG}\ = \frac{\text{Weight gain (g)}}{\text{duration of experiment (days)}} \quad \text{----------} \quad 3.13$$

$$\textbf{SGR} = \frac{lnW_2 - lnW_1}{T_2 - T_1} \times 100 \quad \text{----------} \quad 3.14$$

Where;

W_1 = initia1 weight of fish,

W_2 = fina1 weight of fish and

T = duration of experiment (day).

At the end of the lab phase, five fish were randomly selected from each replicate of the different treatments for the molecular genotypic determination of sex.

5.2.5 **Water quality parameters of fish culture tanks**

Water quality parameters; temperature, dissolved oxygen, pH, ammonia and Nitrite were determined weekly. Dissolved oxygen, Ammonia, Nitrite and pH were measured using Hach® Surface Water Test Kit (Omitoyin *et al.*, 2013). Temperature was measured using a mercury-in-glass thermometer.

5.3 Growth performance of *O. niloticuss*in the pond phase

5.3.1 Experimental procedure

At the end of the laboratory phase, experimental fish were transferred to the Department of Aquaculture and Fisheries fish farm. These fish were placed into hapas (2 m X 1.5 m X 1 m) rigged in earthen based concrete tank according to each treatment and replicates. They were cultured for additional 84 days and fed with commercial diets (CP 40%). The fish were weighed forthnightly and the water quality parameters were monitored and measured. Growth parameters and were monitored and calculated as described in 3.2.4.

At the end of the pond phase, fish were randomly selected from each treatment and replicates for haematology and serum biochemistry.

5.3.2 Water quality parameters

Water quality parameters measured in the pond phase are the same as those described in 3.2.6.

5.4 Haematology and serum biochemistry of *O. niloticuss*

Blood samples from five randomly selected fish (3.44 g) from each replicate of each treatment were collected with capillary tubes from the caudal peduncle by tail ablation fo1lowing pr0cedures of Cockell *et al.* (1992); Murtha *et al.* (2003); Vijayalakshmi *et al.* (2017) and subjected to haematological and serum biochemical analysis following methods of Svobodova *et al.* (1991). Fish were randomly collected from each replicate of each treatment, dried with paper towel and placed on a clean sheet of mackintosh cloth on a table. To collect blood, the caudal peduncle of each fish was severed with a sharp razor blade and capillary tubes were filled. These were emptied into hematocrit tubes gently shaking them to avoid clotting. Collected blood samples were divided in two sets of Eppendorf tubes. One set contained Ethyl diamine tetra acetic acid (EDTA), as anticoagulant, for haematology (hemoglobin, haematocrit and red blood cell counting).

The second set, without anticoagulant, was left to clot at 4^0C and centrifuged at 10000 rpm for 3min at room temperature (27 ^{0}C).

The haematological parameters studied were haemoglobin (Hb), haematocrit or packed cell volume (PCV), erythrocyte or red b1ood ce11 (RBC), leucocyte or white b1ood ce11 (WBC), platelets or thrombocytes, Mean C0rpuscular Vo1ume (MCV), Mean C0rpuscular Haemog1obin C0ncentration (MCHC), Mean C0rpuscular Haemog1obin (MCH) and leucocyte differential count (monocyte,lymphocyte, eosinophil). Haematological analysis was done in the laboratory according to the procedure described by Dein (1984).

5.4.1 White blood cells (WBC) countof *O. niloticus* fed diets with *T. terrestris* extracts

Determination of WBC were done using Hemocytometer (Neubauer) Counting Method. The blood sample collected from each treatment was diluted at a ratio of 1: 20 (blood: 1%HCl as diluting fluid) was used for WBC counts. Blood was drawn to 0.5 mark on a pipette, the pipette was wiped to remove excess blood clinging to the external surface of the pipette, this was followed by drawing haeyem's solution to 110 mark to dilute the blood which was achieved by mixing the blood and dilution fluid through vigorous shaking in a horizontal manner. For WBC, count was determined by drawing blood to 0.5mark of the pipette and 1% HCl to 11 mark to dilute it and shaken vigorously to ensure complete haemolysis of RBC. The blood cells were counted on the counting chamber with the aid of compound microscope.

5.4.2 Haemoglobin determinationof *O. niloticus* fed diets with *T. terrestris* extracts

Haemoglobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI). Hydrochloric acid was taken to the 2 mark of a graduated capillary tube followed by welled mixed unclotted blood by gentle suction. The tube was wiped with a paper towel to remove excess sample on the surface. The blood and HCl mixture

were poured into a graduated tube with distilled water added drop by drop till the colour turned brown matching the brown glass standard in a colorimeter and the Haemoglobin determined using the formula in equation 3.15;

$$\text{Haemoglobin} = \frac{\text{Value obtained}}{100} \times 17.2 \text{ mg/100mL} \quad \text{----------} \quad 3.15$$

5.4.3 Haematocrit of *O. niloticus* fed diets with *T. terrestris* extracts

To determine the haematocrit (HCT) value of the blood sample, heparinized capillary tubes were filled to three quarters with blood and the tube was cleaned with a paper towel and the opposite end was sealed over flame. These tubes were placed in a haematocrit centrifuge for 5 minutes at 10,000 rpm and read on haematocrit reader according to recommendations of Benson *et al.* (1989).

5.4.4 Red blood cell indices determination of *O. niloticus* fed diets with *T. terrestris* extracts

From the results generated for Hb, RBC and HCT, the Mean C0rpuscular Vo1ume (MCV), Mean C0rpuscular Haemog1obin C0ncentration (MCHC), Mean C0rpuscular Haemog1obin (MCH) were calculated (Bain *et al.,* 2006) as shown in equations 3.16 to 3.18;

$$\text{MCV} = \frac{HCT}{RBC} \; X \; 10 \quad \text{----------} \quad 3.16$$

$$\text{MCHC} = \frac{HB}{HCT} \; X \; 100 \quad \text{----------} \quad 3.17$$

$$\text{MCH} = \frac{HB}{RBC} \; X \; 10 \quad \text{----------} \quad 3.18$$

5.4.5 Differential cell countof *O. niloticus* fed diets with *T. terrestris* extracts

Blood samples for biochemical indices were collected into another sample bottle containing no ethylenediamine tetraacetic acid (EDTA). These samples were spurned in the centrifuge at 3,000 rpm and the clearer portion decanted (after centrifugation) into small sample tubes stored in a freezer. Serum biochemical parameters examined included glucose, total protein, albumin, alanine amino transferase (ALT) aspartate amino transferase (AST) and Alanine phosphatase (ALP). Globulin was calculated by subtracting albumin levels from total protein. Serum biochemistry determinations were carried out using commercial test kits, Quimica Clinica Aplicada test kits (QCA, Spain), Randox test kits (Randox, UK) for total proteins and albumin while serum globulin was calculated as the difference between serum total proteins and serum albumin (Colville, 2002). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using spectrophotometric methods (Reitman and Frankel, 1957) and glucose was determined coloremetrically using glucose kits (Trinder, 1969).

5.5 Sex reversal changes in *O. niloticuss* larvae fed with *T. terrestris* extracts at the end of the laboratory phase.

This was carried out at the Biotechnology Laboratory, Department of Animal Science, Faculty of Agriculture, Obafemi Awolowo University, Ife, Nigeria. Sex reversal changes in experimental fish were assessed using electrophoresis method.

5.5.1 Determination of Sex reversal of fish

The qua1ity of DNA was determined by 1.5% agarose ge1 e1ectrophoresis (Salman, 2000).

5.5.2 Marker selection

The primers used in this study were synthesized by INQABA Biotechnological Industries (Pty) Ltd, South Africa. Seven primers were selected from three sex 1inked Sequence-

Characterized Amp1ified Regi0n (SCAR) marker$with the forward and reverse sequences obtained from *Oreochromis niloticuss*(Table 3.2.)(Sun *et al.*,2014). They were employed to ascertain the genotypic sex of the fish.

5.5.3 **Genomic Deoxyribonucleic Acid (DNA) extraction**

Deoxyribonucleic Acid was extracted fr0m sampled fish tissue using ZR Genomic DNA^{TM}-Tissue MicroPrep manufactured by Zymo Research, South Africa according to the manufacturer's instructions. The Proteinase K (Sigma-Aldrich) was prepared by adding 1,040 µl Proteinase K storage Buffer to each Proteinase K tube prior to use and 250 µL β-mercaptoethanol was added to the Genomic Lysis Buffer to obtain a final dilution of 0.5 % (v/v). The tissue sample of 25 mg was added to 95 µL of genomic graded water in a micro-centrifuge tube after which 2X Digestion Buffer (95 µL) and Proteinase K (10 µL) were added. These were then mixed thoroughly and the content in the tube was incubated at 55^0C for three hours in a water bath.

After incubating for three hours, 700 µL Genomic Lysis Buffer was added to the tube and mixed thoroughly by vortexing. The mixture was transferred to a Zymo-Spin^{TM} IIC Column in a collection tube and centrifuged at 10,000 x g for one minute. 200 µL DNA Pre-Wash Buffer was then included to the spin co1umn in a new co1lection tube and spun in a centrifuge at 10,000 x g for one minute after which 400 µL of g-DNA Wash Buffer was combined and further spun at 10,000 x g for another one minute. The spin co1umn was moved to a clean micro-centrifuge tube and 200 µL DNA Elution Buffer was added. This was incubated for 5 minutes at room temperature and centrifuged at top speed for 30

Table 3.2. Selected synthesized primers

Name	Sequence	Bases
Marker 5R F:	TAA ATT AAT GAC ATT TCA GTT ATG	24
Marker 5R R:	CAG AAA TGT AGA CGC CCA GGT ATC	24
Marker 2R F:	AGA CGA TAA ACA GGA CAT GGC TG	23
Reverse-Y:	AAT TTT TTT ACA TAC TGC ACA ATG A	25
Reverse-X:	AAT TTT TTT ACA TAC TGC ACA ATA G	25
Marker 4R F:	AGG AAC CAA GGC GTG ACA GCA GGC	24
Marker 4R R:	GCG CCA ACC ATC ACC ACC GAA CT	23

Source: Sun *et al.* (2014)

seconds to elute the DNA. The eluted DNA were later stored at -8 ^{0}C for molecular analysis (Smith and Morin, 2005).

5.5.4 **Preparation of DNA loading dye**

For 10 ml of 6x DNA loading dye, 25 mg of bromophenol blue is weighed and added to 4 g of sucrose. This was transferred to a screw-capped tube and 7 mL of distilled water was included. The content was mixed until it dissolved completely, the volume was adjusted to 10 ml and mixed thoroughly. The solution turned dark blue with no undissolved particles. The solution was stored at 2 – 8 ^{0}C.

5.5.5 **Preparation of EDTA**

To prepare a stock solution of 0.5 M EDTA, 93.05 g EDTA was dissolved in 400 mL distilled water using a magnetic stirrer and sodium hydroxide was added to adjust the pH until the EDTA dissolves and solution became clear and deionized water was added to adjust the volume to 500 mL (Lee *et al.*, 2012).

3.6.6 **Tris-base, Boric acid and ETDA (TBE) buffer preparation**

To prepare 10X TBE buffer, 108 g TrisBase, 55 g B0ric acid, 9.5 g EDTA were measured into a glass flask and adjusted to a pH of 8.3 with NaOH, the volume was adjusted to 1000 ml by addition of deionized water. 1X TBE was prepared from 10X TBE by measuring 10 ml of 10X TBE into a conical flask and 990ml of distilled water added to it. 1X TBE was used for agarose gel electrophoresis.

3.6.7 **Polymerase Chain Reaction (PCR) products and amplification**

The extracted DNA were amplified using PCR (Simon *et al.*, 1991). The primer mediated amplification of genomic DNA was prepared to a volume of 12.75 µl for PCR amplification as follows:

0.5µl each of forward and reverse primers,

5µl of genomic DNA,

0.5µl nuclease free water and,

6.25 µl Dream Taq Green PCR Master Mix.

The amplification was carried out using Nexus gradient Eppendorf and TECHNE ^{3}Prime/02 thermal cycler. Temperature cycling conditions began with an initial denaturation step of 92 ^{0}C for 5 minutes, followed by 35 cycles of 94 ^{0}C for 10 seconds, 60 ^{0}C for 30 seconds, then a final extension at 72^{0}C for one minute and a hold at 4^{0}C.

3.6.8 **Preparation of agarose gel**

A 1.5% agarose gel was prepared for the PCR electrophoresis. Exactly 0.9 mg agarose was weighed into a 100 mL glass flask and 60 mL of 1X TBE added to it. This was then heated for 90s in a microwave until the agarose had dissolved. The solution was cooled to 60 ^{0}C and 0.5 µl dye, GR Green Nucleic Acid Stain and EZ vision was added and stirred. The solution was then poured into a casting box of the eletropheretic chamber with combs to create the wells. This was allowed to cool and set for 30 minutes before use (Brody and Kern, 2004).

3.6.9 **Polymerase Chain Reaction product electrophoresis and gel documentation**

7.5 µL of the PCR products were analyzed on 1.5% agar0se ge1 e1ectrophoresis pre-stained with GR Green Nucleic Acid Stain at 100V for 30 minutes. Equal volume of 100 bp ready-to-use NEB NO467S Quick-Load DNA Ladder was used as standard molecular weight. Electrophoresis cell (BIO RAD Model No: Mini-Sub® Cell GT) with power supply from THERMO SCIENTIFIC, Model EC300XL2 were set up for electrophoresis while 1X TBE buffer was used for gel preparation and running the electrophoresis. The resulting SCAR patterns were photographed with Canon Powershot G9 into Canon 1250

on a UV High Performance Transilluminator (UVP, Upland CA, USA) and analyzed visually. Comparative analysis of the pooled male and female genomes were according to Sun *et al.*(2014).

5.6 **Statistical analysis**

Data were subjected to descriptive analysis, regression, one – way Analysis of Variance (ANOVA) procedure of SPSS (version 23) and means were separated using the Least Significant Difference (LSD) of the same statistical package. Results for main effects were expressed as mean ± standard error (SE) while results for interaction effects, phytochemical and proximate compositions as mean ± standard deviation (SD).

Amplified bands were scored as binary data (presence as 1 and absence as 0) for homologous bands. The genotype and allele frequencies of fish in each treatment were determined by direct gene counting method (Falconer and Mackay, 1996) and the frequencies were tested for Hardy – Weinberg Equilibrium (HWE) using POPGENE 1 version 32 software package.

CHAPTER FOUR

RESULTS

4.1 Chemical and Nutrient Content Analysis of *Tribulus terrestris*

4.1.1 Phytochemical analysis of *Tribulus terrestris*

The result showed that, except for aqueous at 100 ^{0}C extract, there was no difference in the chemical composition of processed *T. terrestris* extracts relative to solvent used. Qualitative phytochemical screening of *T. terrestris* presented in Table 4.1 revealed that whole *T. terretris* plant, ethanol, ethyl acetate and aqueous (at room temperature, 27 ^{0}C and at 100^{0}C) extracts all tested positive for flavonoid, saponins, anthraquinones, terpenoids and steroids but were negative for tannins and cardiac glycosides. The presence of alkaloids was detected in all extracts except in *T. terrestris* aqueous extract at 100 ^{0}C where it was negative for alkaloid test.

Quantitative screenings of the tested phytochemicals in *T. terrestris* are presented in tables 4.2. The assessment revealed that the present phytochemicals in *T. terrestris*varied significantly ($p < 0.05$) between the whole plant and different extracts. *Tribulus terretris* plant in this study contained 6±0.40 mg/g alkaloid, 2±0.10 mg/g flavonoid, 45±2.00 mg/g saponin and 9±0.20 mg/g steroid, these varied significantly ($p < 0.05$) from the phytochemical contents of the extracts. Alkaloid ranged ($p < 0.05$) from 0.00±0.00 mg/g in *T. terrestris* aqueous (100 ^{0}C) extract to 36±0.50 mg/g in ethyl acetate extract and flavonoid varied ($p < 0.05$) from 6±0.30 mg/g in aqueous (100 ^{0}C) extract to 28±0.50 mg/g in ethyl acetate extract. Saponin levels recorded ranged significantly ($p < 0.05$) from 1±0.00 mg/g in ethyl acetate to 83±0.00 mg/g in aqueous (Rm) extract (TTA_{27}) while steroid varied ($p < 0.05$) from 3±0.00 mg/g in ethyl acetate to 19±1.00 mg/g in ethanol extract (TTE). Saponin content was highest (83±0.00 mg/g) in TTA_{27} followed ($p < 0.05$) by TTE (47±2.30 mg/g) while steroid level was highest (19±1.00 mg/g) in TTE, followed by TTA_{27} (9±0.50 mg/g). These were the two extracts with best saponin and steroid contents.

Table 4.1. Qualitative Analysis of Phytochemical Constituents of *Tribulus terrestris*

Phytochemical	Test	Whole plant	Extracts			
			TTE	TTEA	TTA_{27}	$TTALL_{00}$
Alkaloids	Dragenduff	+	+	+	+	-
	Mayer's	+	+	+	+	-
	Wagner's	+	+	+	+	-
Flavonoids	Dragenduff	+	+	+	+	+
	Mayer's	+	+	+	+	+
	Wagner's	+	+	+	+	+
Saponins	Frothing	++	++	+	++	+
Tannins	ferric chloride	-	-	-	-	-
Anthraquinones	Chloroform/NH_3	+	+	+	+	+
Terpenoids	Chloroform/H_2SO_4	+	+	+	+	+
Cardiac Glycosides	Keller-Killiani	-	-	-	-	-
Steroid	Dragenduff	+	+	+	+	+

Key: ++ = Present and abundant; + = Present and - = Absent

Table 4.2. Qualitative Analysis of Phytochemical Constituents of *Tribulus terrestris* Plant and Extracts

Phytochemical	Whole plant	Extracts			
		TTE	TTEA	TTA_{27}	$TTALL_{00}$
Alkaloids (mg/g)	6.00 ± 0.40^{d}	24.00 ± 1.00^{c}	36.00 ± 0.50^{a}	32.00 ± 0.00^{b}	0.00 ± 0.00^{e}
Flavonoids (mg/g)	2.00 ± 0.10^{e}	20.00 ± 1.50^{b}	28.00 ± 0.50^{a}	11.00 ± 0.50^{c}	6.00 ± 0.30^{d}
Saponins (mg/g)	45.00 ± 2.00^{b}	47.00 ± 2.30^{b}	1.00 ± 0.00^{d}	83.00 ± 0.00^{a}	36.00 ± 1.00^{c}
Steroid (mg/g)	9.00 ± 0.20^{b}	19.00 ± 1.00^{a}	3.00 ± 0.00^{d}	9.01 ± 0.01^{b}	4.00 ± 0.00^{c}

Means with the same superscripts along row are not significantly different ($p>0.05$)

4.1.2 **Proximate analysis of *T. terrestris* plant and extracts**

The proximate composition and gross energy of *T. terrestris* plant and extracts are presented in Table 4.3. The proximate analysis of *T. terrestris* plant and extracts revealed there were significant significant difference ($p < 0.05$) in the nutrient composition between *T. terrestris* whole plant and the extracts, however, there were no difference ($p > 0.05$) between the extracts. The nutrient composition of *T. terrestris* plant used in this study was; moisture 13.59±0.39%, ash composition 24.32±1.50%, ether extract 5.05±0.46% while crude fibre and crude protein were 15.43±0.59% and 17.28±0.04%. The total carbohydrate was 24.34±0.51% but available carbohydrate was 8.91±0.46% and Gross energy of 150.18±2.22 Kcal/g.

The different extracts had varying nutritional composition. Moisture ranged from 68.43±0.06% in aqueous (100 ^{0}C) extract to 65.03±0.06% in ethanol extract, which was not significantly different ($p > 0.05$) from the moisture in aqueous (Rm) extract, while ash varied ($p > 0.05$) was from 14.43±0.51% in aqueous (Rm) extract to 15.30±1.30% in ethanol extract. Ether extract and crude protein were not significantly different ($p > 0.05$) across the different *T. terrestris* extracts which varied from 7.9±0.89% in aqueous (Rm) extract to 8.65±0.31% in ethyl acetate extract and 11.74±0.06% in ethyl acetate extract to 15.75±1.74% in aqueous (Rm) extract respectively. Gross energy was highest in ethanol extract (120.53±4.52 Kcal/g), this was not significantly different ($p > 0.05$) from the gross energy obtained aqueous (Rm) extract and least in ethyl acetate extract with 105.03±2.44 Kcal/g which did not vary from the gross energy in aqueous (100^{0}C) extract.

4.2 **Proximate composition of Experimental diets containing *T. terrestris* extracts**

Table 4.4 showing the results of the nutrient composition of the experimental diets reveal that no significant difference ($p > 0.05$) existed in the nutrient constituents across all the diets containing the *T. terrestris* extract as well as the control diet. The moisture of the diets ranged from 7.26±0.03% to 7.47±0.61%, the ash content of the diets ranged from

Table 4.3. Proximate Composition of *Tribulus terrestris* Plant and Extracts

Parameter	Whole plant	Extracts			
		TTE	TTEA	TTA_{27}	TTA_{100}
Moisture (%)	13.59±0.39[c]	65.03±0.06[b]	69.61±1.09[a]	65.80±0.46[b]	68.43±0.82[a]
Ash (%)	24.32±1.50[a]	15.36±1.30[b]	14.86±0.72[b]	14.43±0.51[b]	14.75±0.70[b]
Ether Extract (%)	5.05±0.46[b]	8.48±0.47[a]	8.65±0.31[a]	7.9±0.89[a]	8.14±0.43[a]
Crude Protein (%)	17.28±0.04[a]	11.9±2.03[b]	11.74±0.06[b]	15.75±1.74[a]	13.04±1.22[b]
Crude Fibre (%)	15.43±0.59[a]	0.04±0.01[b]	0.04±0.03[b]	0.03±0.01[b]	0.04±0.01[b]
Total Carbohydrate (%)	24.34±0.51[a]	-0.8±1.49[b]	-4.89±0.31[c]	-3.90±1.99[c]	-4.41±0.55[c]
Available Carbohydrate (%)	8.91±0.46[a]	-0.84±1.47[b]	-4.93±0.33[c]	-3.93±1.99[c]	-4.43±0.56[c]
Gross Energy (Kcal/g)	150.18±2.22[a]	120.53±4.52[b]	105.03±2.44[c]	118.41±6.43[b]	107.65±2.38[c]

Means with the same superscripts along rows are not significantly different ($p>0.05$)

Table 4.4. Proximate Composition of Experimental Diets Containing *Tribulus terrestris* Extracts

Parameters	MT	0.0	TTA27 (g/kg)					TTE (g/kg)				
			0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Miosture (%)	7.3±0.06[a]	7.26±0.03[a]	7.35±0.01[a]	7.38±0.26[a]	7.39±0.39[a]	7.45±0.7[a]	7.47±0.61[a]	7.35±0.02[a]	7.34±0.03[a]	7.36±0.48[a]	7.43±0.54[a]	7.46±0.58[a]
Ash (%)	6.3±0.01[a]	6.3±0.01[a]	6.29±0.03[a]	6.31±0.11[a]	6.29±0.01[a]	6.28±0.02[a]	6.31±0.03[a]	6.3±0.11[a]	6.31±0.06[a]	6.3±0.08[a]	6.29±0.02[a]	6.31±0.04[a]
Ether extract (%)	4.13±0.04[a]	4.1±0.05[ab]	4.08±0.08[ab]	4.1±0.01[ab]	4.07±0.05[ab]	4.06±0.01[ab]	4.03±0.03[b]	4.08±0.01[ab]	4.1±0.04[ab]	4.08±0.03[ab]	4.07±0.04[ab]	4.03±0.05[b]
Crude Protein (%)	45.56±0.09[a]	45.6±0.14[a]	45.61±0.02[a]	45.6±0.01[a]	45.61±0.10[a]	45.61±0.02[a]	45.6±0.08[a]	45.62±0.04[a]	45.6±0.02[a]	45.61±0.06[a]	45.62±0.01[a]	45.6±0.04[a]
Crude Fibre (%)	5.55±0.02[a]	5.55±0.02[a]	5.56±0.06[a]	5.55±0.01[a]	5.56±0.04[a]	5.56±0.02[a]	5.55±0.01[a]	5.56±0.01[a]	5.56±0.03[a]	5.56±0.01[a]	5.57±0.02[a]	5.55±0.01[a]
Total Carbohydrate (%)	31.16±0.16[a]	31.2±0.09[a]	31.11±0.10[a]	31.06±0.15[a]	31.08±0.31[a]	31.03±0.07[a]	31.04±0.52[a]	31.09±0.10[a]	31.09±0.09[a]	31.09±0.39[a]	31.03±0.57[a]	31.03±0.53[a]
Available Carbohydrate (%)	25.61±0.16[a]	25.65±0.07[a]	25.55±0.14[a]	25.51±0.15[a]	25.52±0.34[a]	25.46±0.71[a]	25.5±0.53[a]	25.53±0.09[a]	25.54±0.11[a]	25.53±0.04[a]	25.46±0.59[a]	25.48±0.54[a]
Gross Energy (Kcal/g)	321.89±0.07[a]	321.87±0.31[a]	321.38±0.26[a]	321.29±0.64[a]	321.16±1.86[a]	320.87±2.81[a]	320.63±2.36[a]	321.34±0.43[a]	321.46±0.14[a]	321.25±1.94[a]	320.92±2.02[a]	320.64±2.16[a]

Means with the same superscripts along rows are not significantly different (p>0.05)

6.28±0.02% to 6.31±0.04% while ether extract varied from 4.06±0.01% to 4.13±0.03%. The crude protein and crude fibre were from 45.56±0.09% to 45.62±0.04% and 5.55±0.01% to 5.57±0.02% respectively. The total available carbohydrate as well as the gross energy of all the diets were also not significantly different. Total and available carbohydrates ranged from 31.03±0.53% to 31.2±0.09% and 25.46±0.59% to 25.65±0.07% respectively while gross energy varied from 320.63±2.36Kcal/g to 321.89±0.07 Kcal/g.

4.3 Growth Performance of *O. niloticus* Fed Diets with *T. terrestris* Extracts

4.3.1 Main effects of extraction and inclusion levels of *Tribulus terrestris* on the growth and survival of *Oreochromis niloticus* for 42 days

The main effects of extract and inclusion levels of *Tribulus terrestris* on the growth performance and survival of *Oreochromis niloticus* in the first 42 days are presented in Table 4.5. There were no significant differences ($p > 0.05$) in the all growth parameters between processing methods of both extracts and inclusion levels. However, the growth indices were slightly higher in *O. niloticus* fed diets containing *T. terrestris* aqueous extract (TTA_{27}) than those fed diets with *T. terrestris* ethanol extract (TTE). Growth response of *O. niloticus* showed an inclusion level dependence on *T. terrestris* extracts in the diets, growth increased with increase in *T. terrestris* extracts in the diets, these were however not significantly different. *Oreochromis niloticus* fed with 0.0 g/kg showed the least growth response and least survival (89.02±0.18%), followed by the group fed with diets containing 0.5 g/kg extracts (89.42±0.18%). The highest growth performance was recorded in fish fed 2.5g/kg followed by fish fed the control diet (MT) while survival was highest in the MT group (90.64±0.18%) followed by those fed with diets containing 2.5g/kg extracts (90.59±0.13%).

Table 4.5. Main Effects of Extract and Inclusion Levels of *T. terrestris* on the Growth and Survival of *O. niloticus*

Parameters	Extraction		±Pooled SE	Inclusion levels							±Pooled SE
	TTA_{27}	TTE		0.0 g/kg	0.5 g/kg	1.0 g/kg	1.5 g/kg	2.0 g/kg	2.5 g/kg	MT	
Initial weight (g)	0.02[a]	0.02[a]	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00
Final weight (g)	0.64[a]	0.60[a]	0.03	0.50[a]	0.52[a]	0.55[a]	0.61[a]	0.67[a]	0.75[a]	0.74[a]	0.06
Weight gain (g)	0.62[a]	0.58[a]	0.03	0.48[a]	0.50[a]	0.53[a]	0.59[a]	0.65[a]	0.73[a]	0.72[a]	0.06
SGR (%/d)	8.20[a]	8.01[a]	0.12	7.66[a]	7.71[a]	7.87[a]	8.12[a]	8.26[a]	8.60[a]	8.50[a]	0.22
Survival (%)	90.09[a]	89.66[a]	0.10	89.02[a]	89.42[a]	89.67[a]	89.80[a]	89.97[a]	90.59[a]	90.64[a]	0.18

Means (±SE) with the same superscripts along rows are not significantly different ($p>0.05$)

4.3.2 Growth performance indices and survival of *O. niloticus*fed diets with*T. terrestris*extractsfor 42 days

Results for growth performance of *O.niloticus* fed diets with varying rates of *T. terrestris* aqueous extract (TTA_{27}) and ethanol extract (TTE) are presented in Tables 4.6 and 4.7, respectively.

Mean weight gain ranged from 0.48±0.04 g to 0.77±0.17 g. Final weight and MWG didn't differ significantly ($p < 0.05$) in fish fed diets that contained 2.5 g/kg TTA_{27} (0.79±0.17 g and 0.77±0.17 g) and MT (0.74±0.24 g and 0.72±0.24 g), there was also no difference between fish fed diets with 1.5 g/kg TTA_{27} (0.63±0.04 g and 0.61±0.04 g) and 2.0 g/kg TTA_{27} (0.71±0.14 g and 0.69±0.14 g) or between fish fed diets with 0.0 g/kg (0.50±0.04 g and 0.48±0.04 g), 0.5 g/kg (0.53±0.10 g and 0.51±0.10 g) and 1.0 g/kg (0.59±0.07 g and 0.57±0.07 g) TTA_{27}, respectively. Similarly, final weight, weight gain and SGR values of fish fed diets containing TTE followed the same significance trends ($p < 0.05$) with those fed diets with TTA_{27}.

Survival percentage ranged from 89.88±0.31% to 91.27±1.18% in fish fed TTA_{27} and from 89.88±0.31% to 90.64±0.35% in fish fed TTE. The result revealed that, survival increased with increasing inclusion levels of *T. terrestris* extracts. In fish fed diets with TTA_{27}, the highest survival rate recorded in those fed diets with 2.5 g/kg did not differ ($p > 0.05$) from the survival of those fed control diets, these however, these rates differed from fish fed diets with other inclusion levels. The least survival rate was in fish fed diets with 0.0 g/kg which was not significantly different from those fed 0.5 g/kg TTA_{27} but was significantly different from survival of fish in all other groups. Fish fed diets with TTE, there were no significant difference ($p>0.05$) in the survival of O. niloticus fed diets containing the varying inclusion levels. However, the highest survival was in the control group followed by those fed diets with 2.5 g/kg TTE. The least survival was recorded in fish fed with 0.0 g/kg diet.

Table 4.6. Growth Response of *O. niloticus* Fed with *T. terrestris* Aqueous Extract for 42 days

Parameters	0.0 (g/kg)	0.5 (g/kg)	1.0(g/kg)	1.5 (g/kg)	2.0 (g/kg)`	2.5 (g/kg)	MT (mg/kg)
Initial weight (g)	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}
Final weight (g)	0.50±0.04^{b}	0.53±0.10^{b}	0.59±0.07^{b}	0.63±0.04^{ab}	0.71±0.14^{ab}	0.79±0.17^{a}	0.74±0.24^{a}
Mean Weight gain (g)	0.48±0.04^{b}	0.51±0.10^{b}	0.57±0.07^{b}	0.61±0.04^{ab}	0.69±0.14^{ab}	0.77±0.17^{a}	0.72±0.24^{a}
SGR (%/d)	7.66±0.21^{c}	7.77±0.50^{c}	8.03±0.30^{bc}	8.21±0.13^{bc}	8.48±0.49^{ab}	8.72±0.54^{a}	8.50±0.78^{ab}
Survival rate (%)	89.02±0.22^{d}	89.84±0.12^{cd}	89.88±0.31^{bc}	89.88±0.19^{bc}	90.08±0.27^{b}	91.27±1.18^{a}	90.64±0.35^{a}

Means (±SE) with the same superscripts along rows are not significantly different (p>0.05)

Table 4.7. Growth Response of *O. niloticus* Fed Diets with *T. terrestris* Ethanol Extract.

Parameters	0.0 (g/kg)	0.5 (g/kg)	1.0 (g/kg)	1.5 (g/kg)	2.0 (g/kg)	2.5(g/kg)	MT (mg/kg)
Initial weight (g)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Final weight (g)	0.50±0.04^{ab}	0.50±0.01^{ab}	0.52±0.12^{ab}	0.59±0.15^{ab}	0.62±0.27^{a}	0.71±0.08^{a}	0.74±0.24^{a}
Mean Weight gain (g)	0.48±0.04^{b}	0.48±0.10^{b}	0.50±0.12^{b}	0.57±0.15^{ab}	0.60±0.27^{ab}	0.69±0.08^{a}	0.72±0.24^{a}
SGR (%/d)	7.66±0.21^{b}	7.65±0.47^{b}	7.71±0.55^{b}	8.02±0.61^{ab}	8.04±1.00^{ab}	8.48±0.27^{a}	8.50±0.78^{a}
Survival rate (%)	89.02±0.22^{a}	89.00±0.62^{a}	89.47±0.11^{a}	89.71±0.35^{a}	89.85±0.23^{a}	89.92±0.46^{a}	90.64±0.35^{a}

Means (±SE) with the same superscripts along rows are not significantly different (p>0.05)

4.3.3 Interaction effects of extract and dietary inclusion levels of *T. terrestris* on the growth and survival of *O. niloticus* for 42 days

The interaction effects of extract and inclusion levels of *T. terrestris* on the growth performance and survival of *O. niloticus* in the first 42 days are presented in table 4.8. All growth parameters measured were significantly different across the extracts and inclusion levels all following a similar trend where final weight and mean weight gain were highest in 2.5 g/kg TTA_{27} (0.79±0.17 g and 0.77±0.17 g) followed by MT (0.74±0.24 g and 0.72±0.24 g); 2.5 g/kg TTE (0.71±0.08 g and 0.69±0.08 g) and 2.0 g/kg (0.71±0.14 g and 0.69±0.14 g) with no significant difference ($p > 0.05$). These inclusion levels also, did not significantly ($p > 0.05$) vary with 1.5 g/kg TTA_{27} (0.63±0.04 g and 0.61±0.04 g) and 2.0 g/kg TTE (0.62±0.27 g and 0.60±0.27 g), however they all varied ($p < 0.05$) in contrast to all other inclusion concentrations of both extracts.

The survival of *O. niloticus* fed diets that have *T. terrestris* extracts were not significantly different ($p < 0.05$) across all treatments. Highest survival rate was recorded in fish fed feed that contained 2.5 g/kg TTA_{27} (91.27±1.18%) followed by those fed the control diet (90.64±0.35%), 2.0 g/kg TTA_{27} (90.08±0.27%) and 2.5 g/kg TTE (89.92±0.46%), respectively. The least survival was in fish fed diets with 0.5 g/kg TTE (89.00±0.62) followed by those fed diet with 0.0 g/kg (89.02±0.22) and 1.0 g/kg TTE (89.47±0.11).

4.3.4 Water Quality Parameters of Fish Culture Water

Mean water quality parameters of the culture environment are presented in Table 4.9. Temperature and pH of the culture water were not significantly varied ($p > 0.05$) across all treatment tanks. Temperature ranged between 24.81±0.56^{0}C and 25.00±0.98^{0}C, while, pH ranged from 7.61±0.03 to 7.7±0.10. Dissolved oxygen varied significantly ($p < 0.05$) was from 4.53±0.07 mg/L to 4.84±0.07 mg/L. Nitrite and ammonia both ranged significantly ($p < 0.05$) from 0.01±0.00 mg/L to 0.02±0.00 mg/L and 0.05 ±0.01 mg/L to 0.1±0.01 mg/L respectively.

Table 4.8 Interaction Effects of Extract and Inclusion Levels of *T. terrestris* on the Growth and Survival of *O. niloticus* for 42 days

parameters	TTA27 (g/kg)								TTE (g/kg)						
	0.0	0.5	1	1.5	2	2.5	MT		0.0	0.5	1	1.5	2	2.5	MT
IW (g)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
FW (g)	0.50±0.04^{a}	0.53±0.10^{a}	0.59±0.07^{a}	0.63±0.04^{a}	0.71±0.14^{a}	0.79±0.17^{a}	0.74±0.24^{a}	0.50±0.04	0.50±0.04^{a}	0.50±0.01^{a}	0.52±0.12^{a}	0.59±0.15^{a}	0.62±0.27^{a}	0.71±0.08^{a}	0.74±0.24^{a}
MWG (g)	0.48±0.04^{a}	0.51±0.10^{a}	0.57±0.07^{a}	0.61±0.04^{a}	0.69±0.14^{a}	0.77±0.17^{a}	0.72±0.24^{a}	0.48±0.04	0.48±0.04^{a}	0.48±0.10^{a}	0.50±0.12^{a}	0.57±0.15^{a}	0.60±0.27^{a}	0.69±0.08^{a}	0.72±0.24^{a}
SGR (%/d)	7.66±0.21^{a}	7.77±0.50^{a}	8.03±0.30^{a}	8.21±0.13^{a}	8.48±0.49^{a}	8.72±0.54^{a}	8.50±0.78^{a}	7.66±0.21	7.66±0.21^{a}	7.65±0.47^{a}	7.71±0.55^{a}	8.02±0.61^{a}	8.04±1.00^{a}	8.48±0.27^{a}	8.50±0.78^{a}
S (%)	89.02±0.22^{c}	89.84±0.12^{bc}	89.88±0.31^{bc}	89.88±0.19^{bc}	90.08±0.27^{abc}	91.27±1.18^{a}	90.64±0.35^{ab}	89.02±0.22	89.02±0.22^{c}	89.00±0.62^{c}	89.47±0.11^{bc}	89.71±0.35^{bc}	89.85±0.23^{bc}	89.92±0.46^{bc}	90.64±0.35^{ab}

Means with the same superscripts along rows are not significantly different (p>0.05)

Table 4.9. Water Quality Parameters of Culture Water for 42 days

Parameter	MT	0.0	TTA_{27} (g/kg)					TTE (g/kg)				
			0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Temperature (^{0}C)	24.99±0.52^{a}	24.91±0.65^{a}	24.81±0.56^{a}	25.00±0.98^{a}	24.98±0.63^{a}	24.90±0.63^{a}	24.87±0.60^{a}	24.91±0.64^{a}	24.76±0.55^{a}	24.83±0.55^{a}	24.88±0.62^{a}	24.84±0.56^{a}
pH	7.61±0.03^{a}	7.66±0.07^{a}	7.67±0.07^{a}	7.7±0.10^{a}	7.68±0.08^{a}	7.67±0.08^{a}	7.66±0.08^{a}	7.63±0.05^{a}	7.64±0.06^{a}	7.66±0.08^{a}	7.66±0.07^{a}	7.65±0.08^{a}
D. O (mg/L)	4.75±0.07^{abc}	4.73±0.06^{abc}	4.58±0.07^{de}	4.84±0.07^{a}	4.68±0.09^{bcd}	4.62±0.09^{cde}	4.71±0.07^{bcd}	4.63±0.06^{cde}	4.80±0.06^{ab}	4.73±0.06^{abc}	4.65±0.05^{cde}	4.53±0.07^{e}
Nitrite (mg/L)	0.01±0.00^{b}	0.01±0.00^{ab}	0.01±0.00^{b}	0.01±0.00^{b}	0.02±0.00^{a}	0.01±0.00^{b}	0.02±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{b}	0.02±0.00^{a}	0.02±0.00^{ab}	0.02±0.00^{ab}
Ammonia (mg/L)	0.06±0.01^{cde}	0.05±0.0le	0.07±0.01^{b}	0.05±0.0le	0.05 ±0.01^{de}	0.06±0.01^{cde}	0.06±0.01^{cde}	0.06±0.01^{bcd}	0.07±0.01^{bc}	0.07±0.01^{b}	0.1±0.01^{a}	0.06±0.01^{cde}

Means with the same superscripts along the column are not significantly different ($p > 0.05$)

4.3.5 **Growth performance indices and survival of *O. niloticus*administered diets that contained *T. terrestris*extractsin the pond**

Results of the growth performance of *O. niloticus* in the pond after the first 42 days are presented in Tables 4.10 and 4.11.

There were significant differences (p α 0.05) in the initial weight, final weight and mean weight gain of *O. niloticus* fed diets containg *T. terrestris* aqueous extracts. The highest initial wight, final weight and MWG were recorded in fish fed diet containg 2.5 g/kg (0.79±0.17 g; 4.90±0.19 g and 4.11±0.31 g) followed but not significantly different (p α 0.05) from those fed control diet MT (0.74±0.24 g; 4.83±0.25 g and 4.09±0.38 g) and 2.0 g/kg (0.71±0.14 g; 4.72±0.22 g and 4.01±0.35 g). Specific growth rate also follows this trend. Survival of *O. niloticus* fed diets containing *T. terrestris* aqueous etracts were not significantly different (p α 0.05) across all groups of fish with the least survival recorded infish fed diets containing 0.0 g/kg *T. terrestris* aquoues extracts.

Oreochromis niloticus fed diets containing *T. terrestris* ethanol extracts had the highest final weight and MWG in fish fed diets containing MT followed by those diets with 2.5 g/kg (4.78±0.02 g and 4.07±0.06 g). Survival in these groups of fish were significantly different (p α 0.05) in fish fed diets containing 0.5 g/kg (89.87±0.62) and 0.0 g/kg (89.89±0.22%) from fish fed diets containing other varying levels of *T. terrestris* ethanol extracts and MT.

Table 4.10. Growth Response of *O. niloticus* Fed with *T. terrestris* Aqueous Extract in the pond

Parameters	0.0 (g/kg)	0.5 (g/kg)	1.0 (g/kg)	1.5 (g/kg)	2.0 (g/kg)	2.5(g/kg)	MT (mg/kg)
Initial weight (g)	0.50 ± 0.04^{c}	0.53 ± 0.10^{c}	0.59 ± 0.07^{bc}	0.63 ± 0.04^{b}	0.71 ± 0.14^{a}	0.79 ± 0.17^{a}	0.74 ± 0.24^{a}
Final weight (g)	2.32 ± 0.16^{c}	2.70 ± 0.10^{c}	3.52 ± 0.24^{b}	3.69 ± 0.28^{b}	4.72 ± 0.22^{a}	4.90 ± 0.19^{a}	4.83 ± 0.25^{a}
Mean Weight gain (g)	1.82 ± 0.19^{c}	2.17 ± 0.08^{bc}	2.93 ± 0.23^{bc}	3.06 ± 0.31^{b}	4.01 ± 0.35^{a}	4.11 ± 0.31^{a}	4.09 ± 0.38^{a}
ADG (g)	0.02 ± 0.00^{c}	0.03 ± 0.01^{bc}	0.04 ± 0.01^{ab}	0.04 ± 0.01^{ab}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
SGR (%/d)	1.83 ± 0.18^{b}	1.96 ± 0.22^{ab}	2.14 ± 0.15^{a}	2.10 ± 0.16^{a}	2.26 ± 0.30^{a}	2.19 ± 0.30^{a}	2.28 ± 0.41^{a}
Survival rate (%)	89.89 ± 0.22^{ab}	90.72 ± 0.13^{a}	90.75 ± 0.31^{a}	90.77 ± 0.19^{a}	90.96 ± 0.27^{a}	91.56 ± 0.50^{a}	91.52 ± 0.36^{a}

Means with the same superscripts along rows are not significantly different ($p>0.05$)

Table 4.11. Growth Response of *O. niloticus* Fed Diets with *T. terrestris* Ethanol Extract in the pond

Parameters	0.0	0.5	1.0	1.5	2.0	2.5	MT
Initial weight (g)	0.50±0.04^{a}	0.50±0.10^{b}	0.52±0.12^{b}	0.59±0.15^{ab}	0.62±0.27^{ab}	0.71±0.08^{a}	0.74±0.24^{a}
Final weight (g)	2.32±0.16^{c}	2.39±0.12^{c}	3.05±0.23^{b}	3.38±0.10^{b}	4.58±0.17^{a}	4.78±0.02^{a}	4.83±0.25^{a}
Mean Weight gain (g)	1.82±0.19^{c}	1.89±0.02^{c}	2.53±0.35^{bc}	2.79±0.17^{bc}	3.96±0.37^{b}	4.07±0.06^{a}	4.09±0.38^{a}
ADG (g)	0.02±0.00^{b}	0.02±0.00^{b}	0.03±0.00^{ab}	0.03±0.00^{ab}	0.05±0.01^{a}	0.05±0.00^{a}	0.05±0.00^{a}
SGR (%/d)	1.83±0.18^{b}	1.87±0.18^{b}	2.13±0.36^{ab}	2.10±0.31^{ab}	2.45±0.52^{a}	2.28±0.13^{ab}	2.28±0.41^{ab}
Survival rate (%)	89.89±0.22^{ab}	89.87±0.62^{b}	90.35±0.10^{ab}	90.59±0.36^{ab}	90.73±0.23^{ab}	90.80±0.47^{ab}	91.52±0.36^{a}

Means with the same superscripts along rows are not significantly different ($p>0.05$)

4.3.6 **Intereaction effects of extract and dietary inclusion levels of *T. terrestris* on the growth and survival of *O. niloticus* after 42 days**

Result of the intereaction effects of *T. terrestris* extracts on the growth and survival of *O. niloticus* in the pond is presented in Table 4.12.

Final weight, Mean weight gain and Specific growth rates were hightest in fish fed diets containing 2.5 g/kg TTA_{27} (4.90±0.19 g; 4.10±0.31 g and 2.19±0.30 %/d) followed by fish fed control diets (4.83±0.25 g; 4.09±0.38 g and 2.28±0.41 %/d), 2.5 g/kg TTE (4.78±0.02 g; 4.07±0.06 g and 2.28±0.13%/d), 2.0 g/kg TTA_{27} and 2.0 g/kg TTE. There were no significantly differences (p α 0.05) in these group of fish. They were however significantly different from fish fed other levels of *T. terrestris* extracts.

Survival of *O.niloticus* fed diets containing *T. terrestris* aqueous and ethanol extracts were not significantly different across all varying inclusion levels except for fish fed diets with 0.5 g/kg TTE and 0.0 g/kg which had the least survival percentages and were significantly different (p α 0.05) from all others.

The optimum mean weight gain for TTA_{27} and TTE is presented in figures 4.1 and 4.2 respectively.

4.3.7 **Water quality parameters of pond**

Table 4.13 shows result of the water quality parameters of the pond where fish were transferred into had temperature ranging from 26.43 ^{0}C to 28.63 ^{0}C and pH of the water ranging from 7.51 to 7.67. The dissolved oxygen of the water ranged from 6.22 mg/L to 9.14 mg/L while nitrite and ammonia values varied from 0.03 mg/L to 0.08 mg/L and 0.06 mg/L to 0.13 mg/L, respectively.

Table 4.12. Interaction Effects of Extract and Inclusion Levels of *T. terrestris* on the Growth and Survival of *O. niloticus* in the pond

Parameters	TTA_{27} (g/kg)								TTE (g/kg)						
	0.0	0.5	1.0	1.5	2.0	2.5	MT		0.0	0.5	1.0	1.5	2.0	2.5	MT
IW (g)	0.50±0.04^{a}	0.53±0.10^{a}	0.59±0.07^{a}	0.63±0.04^{a}	0.71±0.14^{a}	0.79±0.17^{a}	0.74±0.24^{a}	0.50±0.04^{a}	0.50±0.04^{a}	0.50±0.10^{a}	0.52±0.12^{a}	0.59±0.15^{a}	0.62±0.27^{a}	0.71±0.08^{a}	0.74±0.24^{a}
FW (g)	2.32±0.16^{d}	2.70±0.10^{d}	3.52±0.24^{b}	3.69±0.28^{b}	4.72±0.22^{a}	4.90±0.19^{a}	4.83±0.25^{a}		2.32±0.16^{d}	2.39±0.12^{d}	3.05±0.23^{c}	3.38±0.10^{bc}	4.58±0.17^{a}	4.78±0.02^{a}	4.83±0.25^{a}
MWG (g)	1.82±0.19^{d}	2.17±0.08^{c}	2.93±0.23^{bc}	3.06±0.31^{b}	4.00±0.35^{a}	4.10±0.31^{a}	4.09±0.38^{a}		1.82±0.19^{d}	1.89±0.02^{d}	2.53±0.35^{bc}	2.79±0.17^{bc}	3.96±0.37^{a}	4.07±0.06^{a}	4.09±0.38^{a}
ADG (g)	0.02±0.00^{c}	0.03±0.01^{bc}	0.04±0.01^{b}	0.04±0.01^{b}	0.05±0.00^{a}	0.05±0.00^{a}	0.05±0.00^{a}		0.02±0.00^{c}	0.02±0.00^{c}	0.03±0.00^{bc}	0.03±0.00^{bc}	0.05±0.01^{a}	0.05±0.00^{a}	0.05±0.00^{a}
SGR (%/d)	1.83±0.18^{a}	1.96±0.22^{a}	2.14±0.15^{a}	2.10±0.16^{a}	2.26±0.30^{a}	2.19±0.30^{a}	2.28±0.41^{a}		1.83±0.18^{a}	1.87±0.18^{a}	2.13±0.36^{a}	2.10±0.31^{a}	2.45±0.52^{a}	2.28±0.13^{a}	2.28±0.41^{a}
S (%)	89.89±0.22^{b}	90.72±0.13^{b}	90.75±0.31^{b}	90.77±0.19^{b}	90.96±0.27^{b}	91.56±0.50^{a}	91.52±0.36^{a}		89.89±0.22^{b}	89.87±0.62^{b}	90.35±0.10^{b}	90.59±0.36^{b}	90.73±0.23^{b}	90.80±0.47^{b}	91.52±0.36^{a}

Means with the same superscripts along rows not significantly different (p>0.05)

$$MWG = 2.222 + 1.283\ (x) - 0.089\ (x)^2$$

$$R^2 = 0.947$$

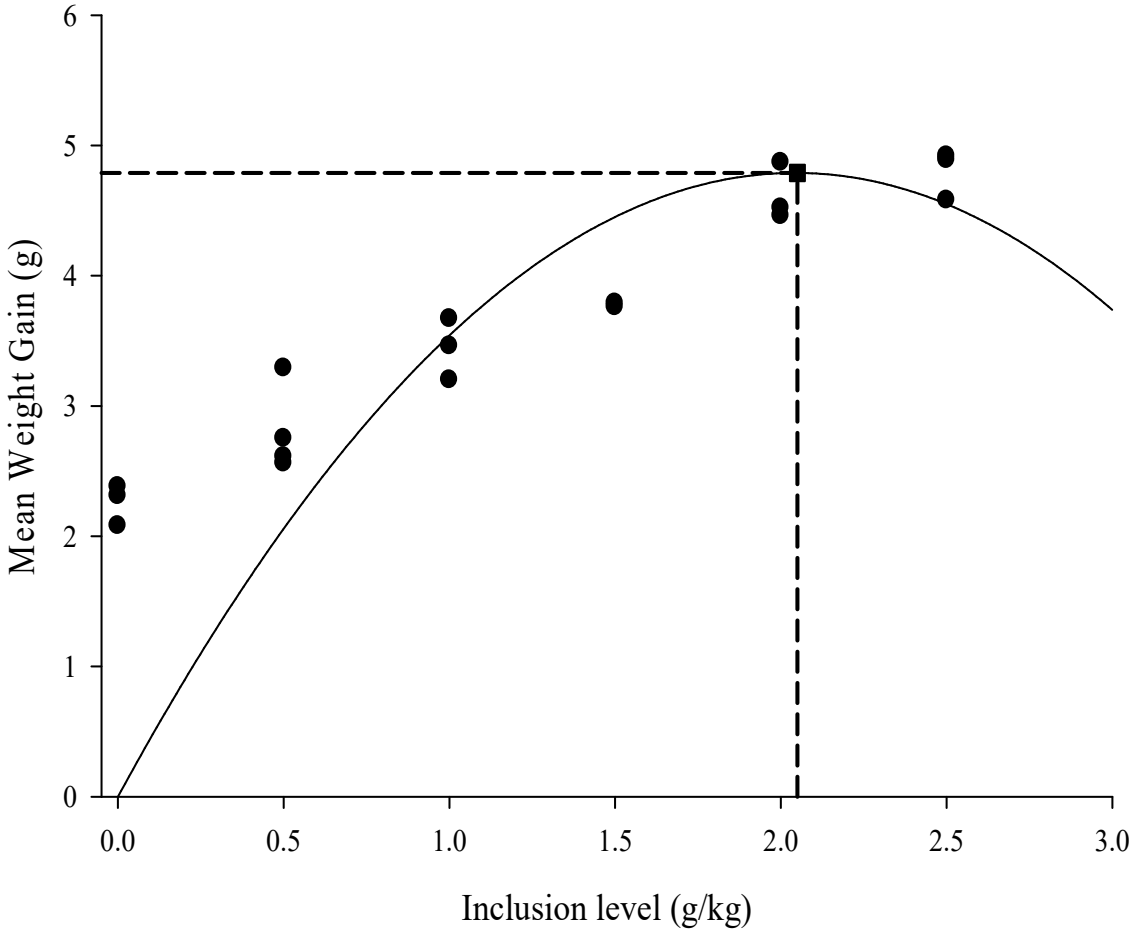

Fig. 4.1. Polynomial regression for mean weight gain for fish fed diet with TTA_{27} extract

Optimum inclusion level of TTA_{27} extract is 2.05 g/kg (Max MWG: 4.79 g)

$$MWG = 2.208 + 0.676\,(x) + 0.148\,(x)^2$$

$$R^2 = 0.917$$

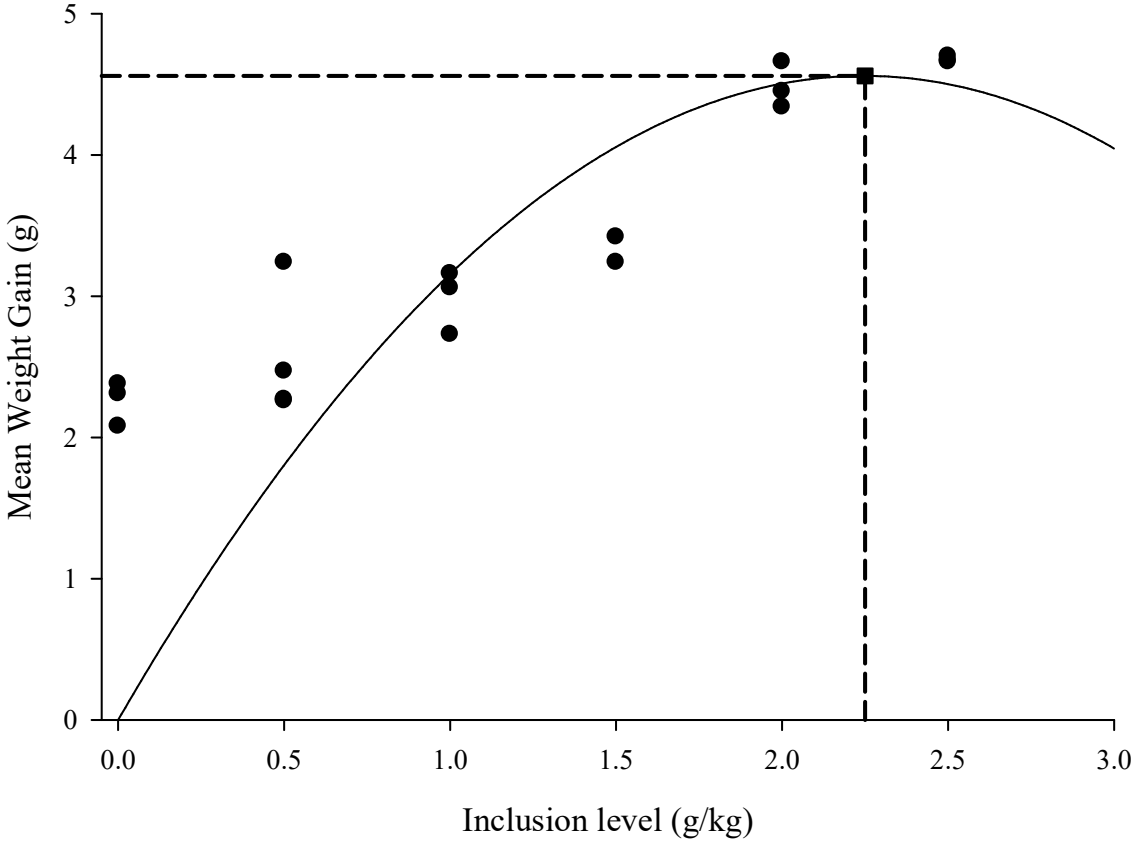

Fig. 4.2. Polynomial regression for mean weight gain for fish fed diet with TTE extract

Optimum inclusion level of TTE is 2.25 g/kg (Max MWG: 4.56 g)

Table 4.13. Water Quality Parameters of Culture Water in the pond

Temperature (^{0}C)	pH	D. O (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)
27.539±1.56	7.59±0.11	7.68±2.06	0.06±0.04	0.10±0.05

7.4 Haematology and serum biochemistry of *O. niloticuss*fed diets containing *T. terrestris* extracts

7.4.1 Main effects of extract and dietary inclusion levels of *Tribulus terrestris* on the haematological response of *Oreochromis niloticuss*

The main effects of *T. terrestris* extracts and the inclusion levels in the diets of *O. niloticuss* are presented in Table 4.14. Results of haematocrit (Hct), Mean Corpuscular Volume (MCV), platelets, White Blood Cell (WBC) and Eosinophils revealed no were not significantly influenced ($p>0.05$) by the extracts. Haemoglobin, Mean Corpuscu1ar Haemog1obin, Mean Corpuscu1ar Haemog1obin Concentrations and lymphocytes were affected significantly ($p < 0.05$) by the different extracts. The haemoglobin, MCH, MCHC and lymphocytes of fish given feeds that had TTE were significantly higher ($p < 0.05$) than the ones given feeds that had TTA_{27}.

Various inclusion rates of*T.terrestris* extracts in the diets fed to *O. niloticuss* had varying degree of influence on the haematological response of the fish. Fish fed control diet MT had the lowest haemoglobin (8.19±0.02 g/dL) which was significantly different from that of those administered feeds that had various amounts of *T. terrestris* extracts. Fish fed diets with 0.5 g/kg *T. terrestris* extracts had the highest haemoglobin (8.67±0.02 g/dL), this diverged significantly ($p < 0.05$) from the ones fed diets containing alternative amounts of *T. terrestris* extracts, however, those fed diets having 2.5g/kg, 2.0 g/kg and 1.5 g/kg *T. terrestris* extracts were not significantly different from those fed diets with 0.0 g/kg. Haematocrit ranged significantly ($p < 0.05$) from 25.20±0.03% in fish fed diets with 2.5g/kg *T. terrestris* extracts to 25.69±0.03% in those fed diets with 0.0 g/kg extracts, however, the haematocrit in fish fed diets with 2.5g/kg extracts was not significantly diverse ($p > 0.05$) fr0m the ones fed control diet and diets that had 1.5 g/kg *T. terrestris* extracts. Those fed diets with 2.5g/kg extract did not have different ($p > 0.05$) haematocrit from those fed the control diet. The WBC count was highest in the control group and least in those fed diets containing 0.5 g/kg *T. terrestris* extracts, however, there existed no significant variation ($p>0.05$) in WBC count of fish given diets containing various amounts of *T. terrestris* extracts and the control diets. Lymphocytes count was highest (91.09±0.12%) in fish fed diet with 0.5 g/kg extracts, this did not differ

($p > 0.05$) from those fed diets with 1.5 g/kg (91.04±0.12%) and 0.0 g/kg (90.90±0.12%) extracts and

Table 4.14. Main Effects of Extract and Inclusion levels of *Tribulus terrestris* on the Haematological Response of *O. niloticus*

Parameters	Extraction		±Pooled SE	Inclusion levels							±Pooled SE
	TTA_{27}	TTE		MT	0.0g/kg	0.5g/kg	1.0g/kg	1.5g/kg	2.0g/kg	2.5 g/kg	
Hb (g/dL)	8.27^{a}	8.43^{b}	0.08	8.19^{a}	8.23^{ab}	8.67^{d}	8.54^{c}	8.24^{ab}	8.26^{ab}	8.29^{b}	0.02
HCT (%)	25.37	25.36	0.01	25.18^{a}	25.69^{d}	25.56^{c}	25.33^{b}	25.20^{a}	25.39^{b}	25.20^{a}	0.03
MCV (fL)	152.81	152.79	0.14	152.65^{b}	150.45^{a}	153.55^{b}	153.74^{b}	152.73^{b}	153.62^{b}	152.86^{b}	0.27
MCH (pg)	49.81^{a}	50.77^{b}	0.05	49.67^{b}	48.22^{a}	52.07^{d}	51.85^{d}	49.94^{bc}	50.00^{bc}	50.30^{c}	0.10
MCHC (%)	32.59^{a}	33.23^{b}	0.04	32.54^{b}	32.05^{a}	33.91^{d}	33.73^{d}	32.70^{bc}	32.55^{b}	32.90^{c}	0.07
Platelets (10^{9}/L)	319.12	319.24	0.09	318.88^{ab}	319.14^{ab}	320.22^{c}	319.32^{b}	319.11^{ab}	319.11^{ab}	318.50^{a}	0.16
WBC (10^{9}/L)	84.43	84.43	0.01	84.49^{b}	84.43^{ab}	84.38^{a}	84.40^{a}	84.40^{a}	84.46^{ab}	84.46^{ab}	0.02
Lymphocytes (%)	90.10^{a}	90.75^{b}	0.06	90.17^{b}	90.90^{c}	91.09^{c}	89.63^{a}	91.04^{c}	90.05^{ab}	90.11^{ab}	0.12
Monocytes (%)	2.53^{b}	2.42^{a}	0.03	2.55^{ab}	2.35^{a}	2.61^{b}	2.55^{ab}	2.43^{ab}	2.41^{ab}	2.44^{ab}	0.05
Eosinophils (%)	1.25	1.25	0.01	1.27^{b}	1.22^{a}	1.25^{ab}	1.24^{ab}	1.25^{ab}	1.25^{ab}	1.25^{ab}	0.01

Means with the same superscripts along rows are not significantly different (p>0.05)

significantly least ($p < 0.05$) in fish fed diet with 1.0 g/kg *T. terrestris* extract (89.63±0.12%) which did not differ ($p > 0.05$) from counts of fish fed diets with 2.5g/kg and control diet. Monocytes and eosinophils counts were highest (2.61±0.05% and 1.27±0.01%) in fish fed diets containing 0.5 g/kg extracts and control diet respectively, these were only significantly increased ($p < 0.05$) than the counts of fish fed diets that contained 0.0 g/kg (2.35±0.05% and 1.22±0.01%).

7.4.1.1 **Haematological parameters of *O. niloticuss* fed diets with *T. terrestris* extracts**

The results for the haematological parameters of fish fed diets containing TTA_{27} and TTE are presented in Tables 4.15 and 4.16, respectively.

Haematological results of fish fed diets containing TTA_{27} showed that fish fed the control diets were not significantly different fr0m fish given feeds that contained TTA27 from 1.5 g/kg. Highest haemoglobin (8.62±0.02 g/dL), MCV (153.87±0.35 fl), MCH (51.69±0.17 pg), MCHC (33.59±0.09%) and platelets (319.90±0.01 x 10^9/L) were obtained in fish fed diets that contain 0.5 g/kg TTA27 extract while, haematocrit (25.73±0.08%) was highest in fish fed diets with 0.0 g/kg extract. However, only MCV was not significantly different ($p>0.05$) fr0m those obtained from fish fed diets that contain other inclusion levels. White blood cell count, monocytes and eosinophils did not significantly differ ($p > 0.05$) across all treatments.

Fish given diets that contained TTE contrarily, showed a trend where, though, the highest haemoglobin (8.71±0.04 g/dL), platelets (320.54±0.47 x 10^9/L) and haematocrit (25.73±0.08%) were obtained in fish fed diets that contain 0.5 g/kg TTE and 0.0 g/kg extract, respectively,highest MCV (153.72±0.42 fl), MCH (52.98±0.45 pg) and MCHC (34.44±0.19%) were obtained in fish fed diets with 1.0 g/kg TTE. These haematological parameters were significantly different in comparison to other experimental considerations.

Table 4.15. Haematological parameters of *O. niloticuss* fed diets with *T. terrestris* aqueous extract

Parameters	Inclusion level of *T. terrestris* aqueous extract (g/kg)						MT (0.5)
	0.0	0.5	1.0	1.5	2.0	2.5	
Hb (g/dL)	8.23±0.02^{c}	8.62±0.02^{a}	8.43±0.03^{b}	8.16±0.17^{de}	8.12±0.17^{e}	8.13±0.17^{e}	8.19±0.04^{cd}
HCT (%)	25.73±0.08^{a}	25.66±0.01^{ab}	25.54±0.04^{b}	25.17±0.01^{c}	25.13±0.06^{c}	25.15±0.05^{c}	25.18±0.03^{c}
MCV (fL)	150.63±0.23^{b}	153.87±0.35^{a}	153.76±0.04^{a}	152.6±0.05^{a}	153.09±0.24^{a}	153.06±0.5^{a}	152.65±0.12^{a}
MCH (pg)	48.22±0.15^{d}	51.69±0.17^{a}	50.72±0.18^{b}	49.44±0.15^{c}	49.46±0.16^{c}	49.47±0.24^{c}	49.67±0.20^{c}
MCHC (%)	32.08±0.14^{d}	33.59±0.09^{a}	32.99±0.13^{b}	32.40±0.1^{c}	32.31±0.08^{c}	32.32±0.06^{c}	32.54±0.11^{c}
Platelets (10^9/L)	319.14±0.04^{bc}	319.90±0.01^{a}	319.22±0.03^{b}	319.35±0.02^{ab}	318.53±0.26^{c}	318.85±0.17^{bc}	318.88±0.62^{bc}
WBC (10^9/L)	84.43±0.02^{a}	84.40±0.06^{a}	84.40±0.08^{a}	84.40±0.05^{a}	84.46±0.002^{a}	84.45±0.003^{a}	84.49±0.01^{a}
Lymphocytes (%)	90.90±0.01^{a}	90.54±0.02^{b}	89.72±0.15^{d}	90.59±0.03^{b}	89.46±0.2^{de}	89.34±0.03^{e}	90.17±0.05^{c}
Monocytes (%)	2.35±0.22^{a}	2.59±0.03^{a}	2.53±0.05^{a}	2.5±0.02^{a}	2.58±0.01^{a}	2.53±0.05^{a}	2.55±0.04^{a}
Eosinophils (%)	1.22±0.03^{a}	1.25±0.023^{a}	1.24±0.02^{a}	1.26±0.00^{a}	1.25±0.023^{a}	1.25±0.11^{a}	1.27±0.01^{a}

Means with the same superscripts along rows are not significantly different at ($p>0.05$)

Table 4.16. Haematological parameters of *O. niloticuss* fed diets with *T. terrestris* ethanol extract

Parameters	0.0	0.5	1.0	1.5	2.0	2.5	MT
Hb (g/dL)	8.23 ± 0.01^{c}	8.71 ± 0.04^{a}	8.67 ± 0.04^{a}	8.32 ± 0.09^{bc}	8.41 ± 0.06^{b}	8.45 ± 0.02^{b}	8.19 ± 0.04^{c}
HCT (%)	25.73 ± 0.08^{a}	25.46 ± 0.03^{b}	25.11 ± 0.02^{c}	25.22 ± 0.14^{c}	25.64 ± 0.11^{ab}	25.24 ± 0.02^{c}	25.18 ± 0.03^{c}
MCV (fL)	150.63 ± 0.23^{b}	153.23 ± 0.17^{a}	153.72 ± 0.42^{a}	152.40 ± 1.05^{a}	154.15 ± 0.11^{a}	152.65 ± 0.50^{a}	152.65 ± 0.12^{a}
MCH (pg)	48.22 ± 0.15^{d}	52.45 ± 0.33^{a}	52.98 ± 0.45^{a}	50.43 ± 0.24^{bc}	50.54 ± 0.20^{b}	51.12 ± 0.26^{b}	49.66 ± 0.20^{c}
MCHC (%)	32.08 ± 0.14^{d}	34.29 ± 0.18^{a}	34.44 ± 0.19^{a}	32.45 ± 0.16^{bc}	32.68 ± 0.11^{c}	33.09 ± 0.09^{b}	32.46 ± 0.11^{cd}
Platelets (10^{9}/L)	319.14 ± 0.04^{abc}	320.54 ± 0.47^{a}	319.43 ± 0.69^{abc}	318.87 ± 0.80^{bc}	319.69 ± 0.34^{ab}	318.15 ± 0.05^{c}	318.88 ± 0.62^{bc}
WBC (10^{9}/L)	84.43 ± 0.02^{ab}	84.37 ± 0.02^{b}	84.4 ± 0.08^{ab}	84.4 ± 0.03^{ab}	84.45 ± 0.04^{ab}	84.46 ± 0.01^{ab}	84.49 ± 0.01^{a}
Lymphocytes (%)	90.90 ± 0.01^{ab}	91.63 ± 0.03^{a}	89.54 ± 0.07^{c}	91.50 ± 0.06^{a}	90.63 ± 0.53^{abc}	90.89 ± 0.88^{ab}	90.17 ± 0.05^{bc}
Monocytes (%)	2.35 ± 0.22^{a}	2.62 ± 0.02^{a}	2.55 ± 0.02^{a}	2.29 ± 0.19^{a}	2.24 ± 0.14^{a}	2.35 ± 0.22^{a}	2.55 ± 0.04^{a}
Eosinophils (%)	1.22 ± 0.03^{a}	1.24 ± 0.03^{a}	1.23 ± 0.04^{a}	1.24 ± 0.02^{a}	1.25 ± 0.01^{a}	1.26 ± 0.00^{a}	1.27 ± 0.01^{a}

Means with the same superscripts along rows are not significantly different at ($p>0.05$)

7.4.1.2 **Interaction effects of extract and dietary inclusion levels of *Tribulus terrestris* extracts on haematological response of *O. niloticuss*.**

The interaction effects of extract and inclusion levels of TTA_{27} and TTE on the haematological parameters of *O. niloticuss* presented in Table 4.17 revealed that there were significant interactions ($p < 0.05$) between the extracts and different inclusion levels in the diets of *O. niloticuss* on the blood parameters except in WBC count, monocytes and eosinophils where there were no significant variations ($p > 0.05$) across all inclusion levels.

Significantly ($p < 0.05$) least haemoglobin was recorded in fish fed MT diet (8.19±0.04 g/dL) which did not differ ($p > 0.05$) from those fed diets that contain 1.5 g/kg TTA_{27}, 2.0 g/kg TTA_{27} and 2.5g/kg TTA_{27} while fish fed 0.5 g/kg TTE had highest haemoglobin (8.71±0.04 g/dL). The same trend observed in the haemoglobin of *O. niloticuss* fed different inclusion amounts of *T.terrestris* extracts was also observed in the levels of Red cell indices (MCH and MCHC) across all treatments. Haematocrit was highest (25.73±0.08%) in fish fed diets with 0.0g/kg *T. terrestris* while the least was obtained in fish fed diets with 2.0 g/kg TTA_{27} (25.13±0.06%), this did not differ ($p > 0.05$) from the haematocrit recorded in 2.5g/kg TTA_{27}, 2.5g/kg TTE and in the control group, MT. The MCV had similar trend as haematocrit in all the treated groups. Platelets count was highest (320.54±0.47 x 10^9/L) in fish fed diets that contain 0.5 g/kg TTE which did not differ ($p > 0.05$) from those fed diets with 0.5 g/kg TTA_{27} (319.90±0.01 x 10^9/L) while, the least (318.15±0.05 x 10^9/L) was obtained in fish fed diets with 2.5g/kg TTE, this did not differ ($p > 0.05$) from those obtained in fish fed diets with 2.5g/kg TTA27 and MT.

4.4.2 **Serum biochemical responses of *O. niloticus* fed diets with *T. terrestris* extracts**

4.4.2.1 **Main effects of extract and inclusion levels of *T. terrestris* extracts on the serum biochemistry of *O. niloticus***

The main effects of extract and dietary inclusion levels of *T. terrestris* extracts on the serum biochemistry of *O. niloticus*are presented in Table 4.18. The result of this study revealed that, extraction methods did not significantly affect ($p > 0.05$) the studied serum

Table 4.17. Interaction effects of extract and dietary inclusion levels of *T. terrestris* extracts on haematological response of *O. niloticuss*.

Parameter	TTA27 (g/kg)							TTE (g/kg)						
	0.0	0.5	1.0	1.5	2.0	2.5	MT	0.0	0.5	1.0	1.5	2.0	2.5	MT
Hb (g/dL)	8.23±0.02^{de}	8.62±0.02^{a}	8.43±0.03^{bc}	8.16±0.17^{e}	8.12±0.17^{e}	8.13±0.17^{e}	8.19±0.04^{e}	8.23±0.02^{de}	8.71±0.04^{a}	8.67±0.04^{a}	8.32±0.09^{cd}	8.41±0.06^{bc}	8.45±0.02^{b}	8.19±0.04^{e}
HCT (%)	25.73±0.08^{a}	25.66±0.01^{a}	25.54±0.04^{ab}	25.17±0.01^{c}	25.13±0.06^{c}	25.15±0.05^{c}	25.18±0.03^{c}	25.73±0.08^{a}	25.46±0.03^{b}	25.11±0.02^{c}	25.22±0.14^{c}	25.64±0.11^{ab}	25.24±0.02^{c}	25.18±0.03^{c}
MCV (fL)	150.63±0.23^{b}	153.87±0.35^{a}	153.76±0.04^{a}	152.6±0.05^{ab}	153.09±0.24^{a}	153.06±0.5^{a}	152.65±0.12^{ab}	150.63±0.23^{b}	153.23±0.17^{a}	153.72±0.42^{a}	152.40±1.05^{a}	154.15±0.11^{a}	152.65±0.50^{ab}	152.65±0.12^{ab}
MCH (pg)	48.22±0.15^{e}	51.69±0.17^{b}	50.72±0.18^{c}	49.44±0.15^{d}	49.46±0.16^{d}	49.47±0.24^{d}	49.66±0.20^{d}	48.22±0.15^{e}	52.45±0.33^{a}	52.98±0.45^{a}	50.43±0.24^{c}	50.54±0.20^{c}	51.12±0.26^{bc}	49.66±0.20^{d}
MCHC (%)	32.08±0.14^{e}	33.59±0.09^{b}	32.99±0.13^{c}	32.40±0.1^{de}	32.31±0.08^{de}	32.32±0.06^{de}	32.46±0.11^{cd}	32.08±0.14^{e}	34.29±0.18^{a}	34.44±0.19^{a}	32.45±0.16^{c}	32.68±0.11^{cd}	33.09±0.09^{b}	32.46±0.11^{cd}
Platelets (10^9/L)	319.14±0.04^{bcd}	319.90±0.01^{ab}	319.22±0.03^{bcd}	319.35±0.02^{bc}	318.53±0.26^{cd}	318.85±0.17^{bcd}	318.88±0.62^{bcd}	319.14±0.04^{bcd}	320.54±0.47^{a}	319.43±0.69^{abc}	318.87±0.80^{bcd}	319.69±0.34^{ab}	318.15±0.05^{d}	318.88±0.62^{bcd}
WBC (10^3/mm^3)	84.43±0.02^{ab}	84.40±0.06^{ab}	84.40±0.08^{ab}	84.40±0.05^{ab}	84.46±0.002^{ab}	84.45±0.003^{ab}	84.49±0.01^{a}	84.43±0.02^{ab}	84.37±0.02^{b}	84.4±0.0^{ab}	84.4±0.03^{ab}	84.45±0.04^{ab}	84.46±0.01^{ab}	84.49±0.01^{a}
Lymphocytes (%)	90.90±0.01^{abc}	90.54±0.02^{cd}	89.72±0.15^{de}	90.59±0.03^{bcd}	89.46±0.2^{e}	89.34±0.03^{e}	90.17±0.05^{cde}	90.90±0.01^{abc}	91.63±0.03^{a}	89.54±0.07^{e}	91.50±0.06^{ab}	90.63±0.53^{bcd}	90.89±0.88^{abc}	90.17±0.05^{cde}
Monocytes (%)	2.35±0.22^{ab}	2.59±0.03^{ab}	2.53±0.05^{ab}	2.5±0.02^{ab}	2.58±0.01^{ab}	2.53±0.05^{ab}	2.55±0.04^{ab}	2.35±0.22^{ab}	2.62±0.02^{a}	2.55±0.02^{ab}	2.29±0.19^{ab}	2.24±0.14^{b}	2.35±0.22^{ab}	2.55±0.04^{ab}
Eosinophils (%)	1.22±0.03^{a}	1.25±0.02^{a}	1.24±0.02^{a}	1.26±0.00^{a}	1.25±0.02^{a}	1.25±0.11^{a}	1.27±0.01^{a}	1.22±0.03^{a}	1.24±0.03^{a}	1.23±0.04^{a}	1.24±0.02^{a}	1.25±0.01^{a}	1.26±0.00^{a}	1.27±0.01^{a}

Means with the same superscripts along rows are not significantly different at (p>0.05)

Table 4.18. Main Effects of Extracts and Inclusion Levels of *T. terrestris* Extracts on the serum Biochemistry of *O. niloticus*

Parameter	Extracts		±Pooled	Inclusion levels							±Pooled
	TTA_{27}	TTE	SE	MT	0.0g/kg	0.5g/kg	1.0g/kg	1.5g/kg	2.0g/kg	2.5 g/kg	SE
AST (µ/L)	64.30	64.14	0.05	64.14^{ab}	64.94^{c}	64.05^{ab}	64.39^{b}	64.34^{b}	63.82^{a}	63.87^{a}	0.10
ALT (µ/L)	129.07	129.33	0.03	128.91^{ab}	129.94^{e}	128.74^{a}	128.95^{ab}	129.49^{d}	129.13^{bc}	129.24^{cd}	0.06
ALP (µ/L)	31.44	31.43	0.04	31.51^{b}	31.13^{a}	31.39^{ab}	31.66^{b}	31.68^{b}	31.14^{a}	31.57^{b}	0.07
ALB (g/dL)	1.52	1.51	0.00	1.51^{a}	1.52^{a}	1.52^{a}	1.51^{a}	1.51^{a}	1.51^{a}	1.53^{a}	0.00
GLO (g/dL)	5.19	5.12	0.03	5.21^{ab}	5.10^{a}	5.00^{a}	5.09^{a}	5.08^{a}	5.22^{ab}	5.36^{b}	0.05
Chol (mg/dL)	141.34	142.08	0.08	162.6of	143.52^{e}	139.38^{d}	137.80^{c}	137.38^{c}	136.17^{b}	135.12^{a}	0.15
Glucose (mg/dL)	96.86	96.95	0.12	97.68^{b}	96.45^{a}	96.65^{a}	96.93^{ab}	96.98^{ab}	96.61^{a}	97.01^{ab}	0.22
Total protein (g/dL)	6.70	6.63	0.03	6.72^{ab}	6.61^{a}	6.52^{a}	6.60^{a}	6.59^{a}	6.73^{ab}	6.89^{b}	0.05

Means with the same superscripts along rows are not significantly different (p>0.05)

biochemistry of *O. niloticus*in the current study. Inclusion level however, significantly influenced ($p < 0.05$) some serum biochemical responses of *O. niloticus*. Albumin, globulin, glucose and total protein levels of *O. niloticus* fed diets that contain the varying inclusion concentrations of *T. terrestris* extracts as well as those fed the control diet, MT revealed not to be significantly different ($p > 0.05$). Highest Aspartate aminotransferase (AST) was recorded from fish fed diets with 0.0 g/kg extracts (64.96±0.10 µ/L), this differed significantly ($p < 0.05$) from AST obtained in fish fed diets that contain all other levels of *T. terrestris* extracts. Fish fed with diets that contain 2.0 g/kg extracts had the least AST (63.82±0.10 µ/L) which were not significantly different from those fed 2.5 g/kg and control diet. Alanine aminotransferase ranged significantly ($p < 0.05$) from 128.74±0.06 µ/L in fish fed with diets

4.4.2.2 Serum biochemical parameters of *O. niloticus* fed diets with *T. terrestris* extracts

Tables 4.19 and 4.20 show the results of the serum biochemistry of fish fed with diets that contain TTA_{27} and TTE, respectively. Serum biochemistry of *O. niloticus* fed with diets that contain TTA_{27} and TTE extracts were all significantly different ($p > 0.05$) across all different inclusion levels except in the levels of globulin and glucose which were not significantly different ($p > 0.05$).

Aspartate aminotransferase levels ranged significantly ($p < 0.05$) from 63.71±0.02 µ/L in fish fed diets with 2.5 g/kg TTA_{27} to 64.94±0.04µ/L in fish fed diets with 0.0g/kg*T. terrestris* extract. The level of AST in fish fed diets with TTA_{27} presented a level-dependent decrease with increase in *T. terrestris* extract inclusion level, however, there was no difference ($p < 0.05$) in fish fed diets with 2.0 g/kg and 2.5 g/kg TTA_{27} but these had AST levels significantly different from AST obtained from fish fed diets with other level of TTA_{27} and the control diet. Fish fed with TTE on the hand had AST levels ranging ($p < 0.05$) from 63.57±0.58 µ/L in fish fed diets with 0.5 g/kg TTE to 64.94±0.04µ/L in fish fed diets with 0.0 g/kg extract. The AST levels were significantly

different in fish fed diets with varying levels of TTE however, fish fed diets that contain 2.5 g/kg were

Table 4.19. Serum Biochemical Parameters of *O. niloticus* Fed Diets Containing *T. terrestris* Aqueous Extract

Parameters	MT	0.0	0.5	1.0	1.5	2.0	2.5
AST (µ/L)	64.14±0.04^{c}	64.94±0.04^{a}	64.52±0.03^{b}	64.93±0.02^{a}	64.11±0.03^{c}	63.73±0.15^{d}	63.71±0.02^{d}
ALT(µ/L)	128.91±0.18^{c}	129.94±0.02^{a}	128.55±0.04^{e}	128.70±0.02^{de}	129.51±0.05^{b}	128.89±0.02^{cd}	128.98±0.03^{c}
ALP (µ/L)	30.51±0.03^{b}	31.13±0.11^{c}	31.48±0.04^{b}	31.47b±0.03^{b}	31.56±0.06^{b}	31.16±0.04^{c}	31.81±0.03^{a}
ALB (g/dL)	1.52±0.01^{ab}	1.52±0.01^{ab}	1.52±0.02^{ab}	1.51±0.01^{b}	1.50±0.01^{b}	1.52±0.01^{ab}	1.54±0.01^{a}
GLO (g/dL)	5.21±0.02^{a}	5.10±0.25^{a}	5.08±0.09^{a}	5.12±0.14^{a}	5.11±0.04^{a}	5.30±0.04^{a}	5.39±0.16^{a}
Chol (mg/dL)	162.60±0.33^{a}	143.52±0.04^{b}	138.87±0.26^{c}	137.40±0.47^{d}	136.91±0.11^{d}	135.66±0.44^{e}	134.44±0.16^{f}
Glucose (mg/dL)	97.68±0.51^{a}	96.45±0.58^{a}	96.71±0.10^{a}	96.99±0.61^{a}	96.78±0.61^{a}	96.94±0.76^{a}	97.03±0.53^{a}
Total protein (g/dL)	6.72±0.01^{a}	6.61±0.25^{a}	6.59±0.07^{a}	6.63±0.14^{a}	6.62±0.05^{a}	6.82±0.04^{a}	6.90±0.15^{a}

Means with the same superscripts along rows arenot significantly different (p>0.05)

Key:

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

ALP: Alkaline phosphate

ALB: Albumin

GLO: globulin

Table 4.20. Serum Biochemical Parameters of *O. niloticus* Fed Diets Containing *T. terrestris* Ethanol Extract

Parameters	MT	0.0	0.5	1.0	1.5	2.0	2.5
AST (µ/L)	64.14±0.04^{abc}	64.94±0.04^{a}	63.57±0.58^{c}	63.85±0.63^{bc}	64.56±0.11^{ab}	63.91±0.14^{bc}	64.02±0.02^{abc}
ALT (µ/L)	128.91±0.18^{c}	129.94±0.02^{a}	128.93±0.36^{c}	129.21±0.03^{bc}	129.46±0.20^{ab}	129.37±0.10^{bc}	129.50±0.08^{ab}
ALP (µ/L)	31.51±0.03^{ab}	31.13±0.11^{b}	31.30±0.23^{ab}	31.85±0.04^{a}	31.79±0.22^{a}	31.11±0.12^{b}	31.32±0.51^{ab}
ALB (g/dL)	1.51±0.01^{a}	1.52±0.01^{a}	1.51±0.01^{a}	1.51±0.01^{a}	1.51±0.02^{a}	1.51±0.01^{a}	1.51±0.02^{a}
GLO (g/dL)	5.21±0.01^{ab}	5.10±0.25^{ab}	4.93±0.21^{b}	5.05±0.11^{ab}	5.05±0.04^{ab}	5.12±0.01^{ab}	5.36±0.06^{a}
Chol (mg/dL)	162.60±0.33^{a}	143.52±0.04^{b}	139.8±0.08^{c}	138.21±0.12^{d}	137.84±0.15^{de}	136.69±0.37^{ef}	135.79±1.05^{f}
Glucose (mg/dL)	97.68±0.51^{a}	96.45±0.58^{a}	96.59±0.89^{a}	96.88±0.10^{a}	97.18±0.03^{a}	96.89±0.55^{a}	96.98±0.08^{a}
Total protein (g/dL)	6.72±0.01^{ab}	6.61±0.25^{ab}	6.44±0.29^{b}	6.56±0.12^{ab}	6.56±0.06^{ab}	6.63±0.02^{ab}	6.87±0.07^{a}

Means with the same superscripts along rows are notnot significantly different (p>0.05

not significantly different ($p > 0.05$) compared to the ones fed contro, MT. Alanine aminotransferase significantly varied ($p < 0.05$) from 128.55±0.04 µ/L and 128.91±0.18 µ/L in fish fed with 0.5 g/kg TTA_{27} to 129.94±0.02 µ/L in fish fed diets with 0.0 g/kg as with the AST levels, fish fed diets with 2.5 g/kg TTA_{27} did not differ ($p > 0.05$) from those fed diets with 2.0g/kg and control diet. Least ALT in fish fed diets with TTE was obtained in fish fed control diets (128.91±0.18µ/L) which was not significantly different from the ALT of fish fed diet with 0.5 g/kg TTE but differed significantly ($p < 0.05$) from those fed 2.5 g/kg TTE. Mean values of albuminin fish fed diets with 2.5 g/kg TTA_{27} were different ($p < 0.05$) from those fed diets with 1.0 g/kg TTA_{27} and 1.5 g/kg TTA_{27} but was not different ($p > 0.05$) from fish fed control diet and other inclusion levels. Fish fed diets with TTE however, had significantly similar ($p > 0.05$) albumin levels across all inclusion levels.

Serum cholesterol (SC) level were significantly different across fish fed feeds that contained all inclusion levels of *T. terrestris* extracts. In fish fed diets with both TTA_{27} and TTE extracts, the least serum cholesterol levels were recorded in fish fed diets with 2.5 g/kg TTA_{27} (134.44±0.16 mg/dL) and 2.5 g/kg TTE (135.79±1.05 mg/dL), respectively while, maximum SC in this study was recorded in fish fed control, MT (162.60±0.33 mg/dL). There was a decrease in cholesterol level with increase in inclusion levels though this was significantly different ($p < 0.05$) across the various groups. Mean values of Glucose were also not significantly different across the treatments with the least value in fish fed diets with 0.0 g/kg (96.45±0.58 g/dL) and the highest in fish fed with control diet, MT (97.68±0.51 g/dL) followed by those fed diets that contain 2.5 g/kg TTA_{27} (97.03±0.53 g/dL) and 1.5 g/kg TTE (97.18±0.03 g/dL) respectively. Total protein mean values across the treatments showed significant difference ($p < 0.05$) only in fish fed diets with 2.5 g/kg of both extracts and had values ranging from 6.59±0.07 g/dL to 6.90±0.15 g/dL in fish fed diets with TTA_{27} extracts and from 6.44±0.29 g/dL to 6.72±0.01 g/dL in fish fed diets with TTE extracts, respectively. There were however, no significance difference ($p > 0.05$) between fish fed diets with the extracts and those fed the control diets.

4.4.2.3 **Interaction effects of extraction and inclusion levels of *T. terrestris* on the serum biochemistry of *O. niloticus***

The results of the interaction effects between extracts and inclusion levels of *T. terrestris* extracts on the serum biochemistry of *O. niloticus* are presented in Table 4.21. The results revealed that, there exists no significant interaction ($p > 0.05$) between inclusion levels and extracts as it relates to albumin, globulin, glucose and total protein levels found in the fish in the current study. The highest AST (64.94±0.04 µ/L) was recorded in fish fed diets with 0.0 g/kg extracts which were not different ($p > 0.05$) from those recorded in fish fed diets that contain 1.0 g/kg TTA_{27} 0.5 g/kg TTA_{27} and 1.5 g/kg TTE. These were however different ($p < 0.05$) from those fed diets with 2.5 g/kg TTA_{27} which had the least (63.71±0.02 µ/L) AST and was not significantly different ($p > 0.05$) from the ones obtained in fish of the MT group and those fed diets with 2.5 g/kg TTE. The ALT significantly ranged ($p < 0.05$) from 128.55±0.04 µ/L in fish fed diets with 0.5 g/kg TTA_{27} to 129.94±0.02 µ/L in fish fed diets with 0.0 g/kg extracts. The ALT recorded in fish fed control diet was not significantly different ($p > 0.05$) from the ones fed diets with 2.0 g/kg TTA_{27} and 2.5 g/kg TTA_{27}. Serum cholesterol was highest (162.60±0.33 g/dL) in fish fed control diet, MT which showed significant variation ($p < 0.05$) from fish fed feeds with 0.0 g/kg (143.52±0.04 mg/dL) and all levels of inclusion of TTA_{27} and TTE extracts. The serum cholesterol level however, decreased with increasing inclusion level of *T. terrestris* extracts with fish fed in diets that contain 2.5 g/kg TTA_{27} having the least (134.44±0.16 mg/dL) serum cholesterol level.

4.5 **Sex Reversal Changes in *O. niloticus* Larvae Fed Diets containing *T. terrestris*Extracts**

There were significant variations in the effects of *T. terrestris* extracts on the sex ratios and genotypic changes in treated *O. nilotiucus* larvae.

Table 4.21. Interaction Effects of Extracts and Inclusion Levels of *T. terrestris* on serum Biochemistry of *O. niloticus*

Parameter	MT	0.0	TTA_{27} (g/kg)							T
			0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5
AST (µ/L)	64.14±0.04^{bc}	64.94±0.04^{a}	64.52±0.03^{ab}	64.93±0.02^{a}	64.11±0.03^{bc}	63.73±0.15^{c}	63.71±0.02^{c}	63.57±0.58^{c}	63.85±0.63^{bc}	64.
ALT (µ/L)	128.91±0.18^{def}	129.94±0.02^{a}	128.55±0.04^{f}	128.70±0.02^{ef}	129.51±0.05^{b}	128.89±0.02^{def}	128.98±0.03^{cde}	128.93±0.36^{def}	129.21±0.03^{bcd}	129
ALP (µ/L)	31.51±0.03^{abc}	31.13±0.11^{c}	31.48±0.04^{abc}	31.47b±0.03^{abc}	31.56±0.06^{abc}	31.16±0.04^{c}	31.81±0.03^{ab}	31.30±0.23^{bc}	31.85±0.04^{a}	31.
ALB (g/dL)	1.51±0.01^{ab}	1.52±0.01^{ab}	1.52±0.02^{ab}	1.51±0.01^{ab}	1.50±0.01^{b}	1.52±0.01^{ab}	1.54±0.01^{a}	1.51±0.01^{ab}	1.51±0.01^{ab}	1.5
GLO (g/dL)	5.21±0.01^{ab}	5.10±0.25^{ab}	5.08±0.09^{ab}	5.12±0.14^{ab}	5.11±0.04^{ab}	5.30±0.04^{a}	5.39±0.16^{a}	4.93±0.21^{b}	5.05±0.11^{ab}	5.0
Chol (mg/dL)	162.60±0.33^{a}	143.52±0.04^{b}	138.87±0.26^{cd}	137.40±0.47^{ef}	136.91±0.11^{fg}	35.66±0.44^{h}	134.44±0.16^{i}	139.8±0.08^{c}	138.21±0.12^{de}	137
Glucose (mg/dL)	97.68±0.51^{a}	96.45±0.58^{a}	96.71±0.10^{a}	96.99±0.61^{a}	96.78±0.61^{a}	96.94±0.76^{a}	97.03±0.53^{a}	96.59±0.89^{a}	96.88±0.10^{a}	97.
Total protein (g/dL)	6.72±0.01^{ab}	6.61±0.25^{ab}	6.59±0.07^{ab}	6.63±0.14^{ab}	6.62±0.05^{ab}	6.82±0.04^{a}	6.90±0.15^{a}	6.44±0.29^{b}	6.56±0.12^{ab}	6.5

Means with the same superscripts along rows are not significantly different (p>0.05)

4.5.1 Allele and genotype frequencies of *O. niloticus* fed diets with *T. terrestris* extracts

Results for the allele and genotype frequencies of fish fed diets that contain TTA_{27} and TTE are presented in Tables 4.22 and 4.23, respectively. Two alleles (X and Y) and two genotypes (XX and XY) were observed after running gel Electrophoresis and viewed under the UV light in a trans-illuminator. The total frequencies of 0.5556 (X), 0.4444 (Y) and 0.5722 (X), 0.4278 (Y) alleles were observed for fish fed diets that contain TTA_{27} and TTE, respectively. The total genotypic frequencies for XX and XY of *O. niloticus* fed diets with TTA_{27} and TTE extracts were 0.11 and 0.89, respectively. Chi - square tests for Hardy-Weinberg equilibrium revealed that there were significance differences ($p>0.05$) for fish fed diets with 0.5 g/kg and 1.0 g/kg and ($p>0.01$)for fish fed feeds that had 1.5 g/kg, 2.0 g/kg, 2.5 g/kg extracts, same as fish fed control diet, MT. These significant differences indicate a deviation from the Hardy-Weinberg expected genotype frequencies.

Total genic variation result presented in Table 4.24 revealed that there were no difference among fish fed diets that contain different inclusion concentration of TTA_{27} and TTE indicating that, there is a close genetic relationship in the studied fish as the observed alleles (na = 2.000) was almost the same for the effective number of alleles [ne = 1.9756 (TTA_{27}); 1.9591 (TTE)]. Degree of diversity was however noticed to increase with increasing inclusion levels of *T. terrstris* extracts with the lowest recorded in fish fed diets that contain 0.0 g/kg (ne = 1.6423; I = 0.5799) and the highest recorded in fish fed diets with 2.5 g/kg (ne = 2.000; I = 0.6931). The Shannon information index showed that most of the loci were highly informative indicating the polymorphism across the loci.

Tables 4.25 and 4.26 show the results for the heterozygosity of fish fed diets that contain TTA_{27} and TTE extracts, respectively. Fish in all treatments had higher observed heterozygosity ranging from 0.5333 to 1.000 compared to the expected heterozygosity that ranged from 0.4046

Table 4.22. Allele and Genotype Frequencies for *O. niloticus* Fed Diets with *T. terrestris* Aqueous Extracts

Inclusion level (g/kg)	Allele	Frequency	Genotype	Frequency	X^2
0.0	X	0.7333	XX	0.47	1.6970
	Y	0.2667	XY	0.53	
0.5	X	0.5667	XX	0.13	8.0294**
	Y	0.4333	XY	0.87	
1.0	X	0.5333	XX	0.07	10.6167**
	Y	0.4667	XY	0.93	
1.5	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
2.0	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
2.5	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
MT	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
Total	X	0.5556	XX	0.11	56.8162
	Y	0.4444	XY	0.89	

X^2 = Chi-square test for Hardy-Weinberg equilibrium

** = significance at $p < 0.01$

*** = significance at $p < 0.001$.

Table 4.23. Allele and Genotype Frequencies for *O. niloticus* Fed Diets with *T. terrestris* Ethanol Extracts

Inclusion level (g/kg)	Allele	Frequency	Genotype	Frequency	X^2
0.0	X	0.7333	XX	0.47	1.6970
	Y	0.2667	XY	0.53	
0.5	X	0.6000	XX	0.20	6.0392*
	Y	0.4000	XY	0.80	
1.0	X	0.5667	XX	0.13	8.0294**
	Y	0.4333	XY	0.87	
1.5	X	0.5333	XX	0.07	10.6167**
	Y	0.4667	XY	0.93	
2.0	X	0.5000	XX	0.00	14.0000**
	Y	0.5000	XY	1.00	
2.5	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
MT	X	0.5000	XX	0.00	14.0000***
	Y	0.5000	XY	1.00	
Total	X	0.5722	XX	0.11	49.5743
	Y	0.4278	XY	0.89	

X^2 = Chi-square test for Hardy-Weinberg equilibrium

** = significance at $p < 0.01$

*** = significance at $p < 0.001$.

Table 4.24. Genic Variation of *O. niloticus* Fed Diets with *T. terrestris* Extracts

Inclusion level (g/kg)	Sample Size	na*	Aqueous		Ethanol	
			ne*	I*	ne*	I*
0.0	15.00	2.00	1.6423	0.5799	1.6423	0.5799
0.5	15.00	2.00	1.9651	0.6842	1.9231	0.6730
1.0	15.00	2.00	1.9912	0.6909	1.9651	0.6842
1.5	15.00	2.00	2.0000	0.6931	1.9912	0.6909
2.0	15.00	2.00	2.0000	0.6931	2.0000	0.6931
2.5	15.00	2.00	2.0000	0.6931	2.0000	0.6931
MT	15.00	2.00	2.0000	0.6931	2.0000	0.6931
Total	90.00	2.00	1.9756	0.6870	1.9591	0.6827

na = Observed number of alleles

ne = Effective number of alleles

I = Shannon's Information index

Table 4.25. Heterozygosity of *O. niloticus* Fed Diets with *T. terrestris*Aqueous Extract

Inclusion level (g/kg)	Sample size	Observed Homozygosity	Observed Heterozygosity	*Expected Homozygosity	*Expected Heterozygosity	Nei**	Average Heterozygosity
0.0	15.00	0.4667	0.5333	0.5954	0.4046	0.3911	0.4800
0.5	15.00	0.1333	0.8667	0.4920	0.5080	0.4911	0.4800
1.0	15.00	0.0667	0.9333	0.4851	0.5149	0.4978	0.4800
1.5	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.4800
2.0	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.4800
2.5	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.4800
MT	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.5000
Total	90.00	0.1111	0.8889	0.5034	0.4966	0.4938	0.4800

* Expected homozygosity and observed heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

Table 4.26. Heterozygosity of *O. niloticus* Fed Diets with *T. terrestris*Ethanol Extract

Inclusion level (g/kg)	Sample size	Observed Homozygosity	Observed Heterozygosity	*Expected Homozygosity	*Expected Heterozygosity	Nei**	Average Heterozygosity
0.0	15.00	0.4667	0.5333	0.5954	0.4046	0.3911	0.4767
0.5	15.00	0.2000	0.8000	0.5034	0.4966	0.4800	0.4767
1.0	15.00	0.1333	0.8667	0.4920	0.5080	0.4911	0.4767
1.5	15.00	0.0667	0.9333	0.4851	0.5149	0.4978	0.4767
2.0	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.4767
2.5	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.4767
MT	15.00	0.0000	1.0000	0.4828	0.5172	0.5000	0.5000
Total	90.00	0.1444	0.8556	0.5077	0.4923	0.4896	0.4767

* Expected homozygosity and observed heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

to 0.5172. The total observed heterozygosity of 0.8889 was markedly higher that the expected heterozygosity of 0.4966 with a deviation 0.4938 factor from Nei's expected heterozygosity for *O. niloticus*fed diets that contain different levels of TTA_{27} extracts (Table 4.23) and a total observed heterozygosity of 0.8556 against the expected heterozygosity of 0.4923 with a deviation 0.4896 factor from Nei's expected heterozygosity for *O. niloticus*fed diets that contain TTE extracts (Table 4.24). This indicates a higher genetic variability within the fish in each treatment. Consequently, the observed homozygosity was lower than those of the expected homozygosity.

4.5.2 Main effects of extract and inclusion levels of *T. terrestris* extracts on sex reversal changes of *O. niloticus*

The main effects of extract and inclusion levels of *T. terrestris* extracts on sex reversal changes of *O. niloticus* are shown in Table 4.27. Results for main effects of extracts on the sex reversal of *O. niloticus* show that there was no significant variation ($p > 0.05$) between the effects *T. terrestris* aqueous (Rm) extract (TTA_{27}) and *T. terrestris* ethanol extract (TTE). Numerically, fish fed diets that contain TTA_{27} extracts had a higher percentage of males and lower percentage of females compared to their counterparts fed diets with TTE extracts, respectively. Based on inclusion levels of *T. terrestris* extracts, significant effect ($p < 0.05$) was observed between fish fed diets with 0.0 g/kg extracts and those fed the control diet and those fed other levels of *T. terrestris* extracts. The sex reversal changes in the control group was however, not different ($p > 0.05$) from the sex reversal changes of fish fed diets of other inclusion levels of *T. terrestris* extracts.

4.5.3 Sex ratio of *O. niloticus* fed diets with varying levels of *T. terrestris* extracts

Results of percentage males of *O. niloticus* fed diets with various inclusion levels of *T. terrestris* aqueous (TTA_{27}) and ethanol (TTE) extracts are presented in figure 4. 3 and the results of sex ratio of *O. niloticus* fed diets that contain *T. terrestris* extracts are presented in figure 4.18. The results showed an increase in percentage males in all groups of fish

Table 4.27. Main Effects of Extraction and Inclusion Levels of *T. terrestris* Extracts on Sex Reversal Changes of *O. niloticus*

Sex ratio	Extract		Pooled SE	Inclusion level							±Pooled SE
	TTA_{27}	TTE		MT	0.0g/kg	0.5g/kg	1.0g/kg	1.5g/kg	2.0g/kg	2.5 g/kg	
Females (%)	9.52	12.38	2.02	0.00^{a}	46.67^{b}	16.67^{a}	10.00^{a}	3.33^{a}	0.00^{a}	0.00^{a}	3.78
Males (%)	90.48	87.62	2.02	100.00^{b}	53.33^{a}	83.33^{b}	90.00^{b}	96.67^{b}	100.00^{b}	100.00^{b}	3.78

Means with the same superscripts along rows are not significantly different ($p>0.05$)

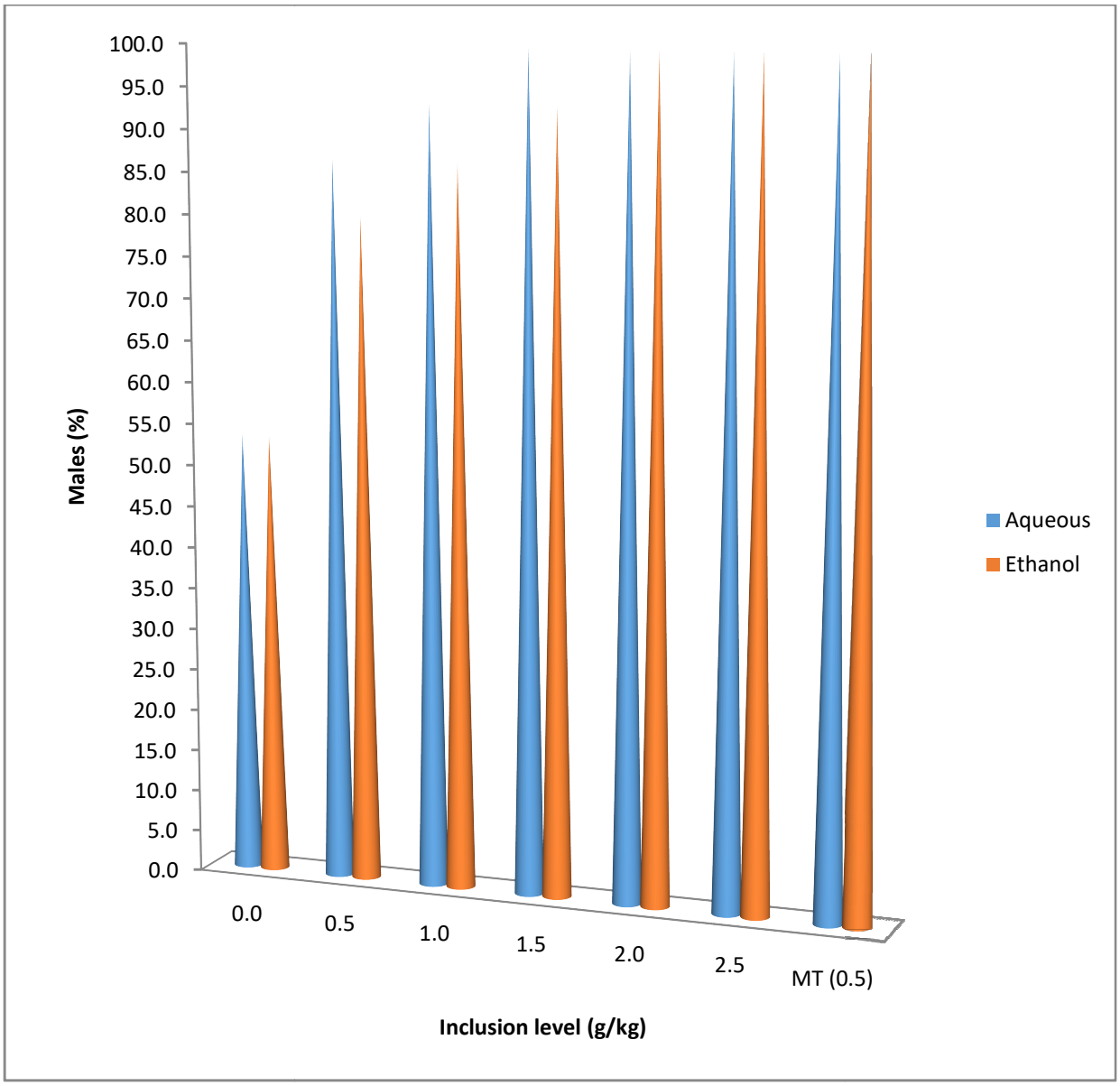

Fig. 4.3. Percentage male *O. niloticus* fed diets containing TTA_{27} and TTE extracts

with increasing inclusion levels of TTA_{27} and TTE (fig. 4. 17). There was no significance differences ($p > 0.05$) in the percentage males and females in *O. niloticus* fed diets that contain TTA_{27} and TTE extracts as well as no variation ($p > 0.05$) from those fed the control diet, MT. An all-male population was recorded in this study which is similar to the results of Kiriyakit (2014) who tested the efficacy of *Butea superb* as sex reversal agent and recorded a 100% sex reversal of *O. niloticus*. Fish fed control diet, MT and in fish fed diets that contain TTA_{27} extract from 1.5 g/kg and TTE extracts from 2.0 g/kg and least males (53.33±11.55%) was recorded in fish fed diets that contain 0.0 g/kg extract. Similarly, percentage females decreased with increase in *T. terrestris* extracts with the highest percentage females (46.67±11.55%) obtained in fish fed diets that contain 0.0 g/kg extracts.

4.5.4 Interaction effects of extract and inclusion levels of *T. terrestris* extracts on sex reversal changes in *O. niloticus*

The interaction effects of extract and inclusion levels of *T. terrestris* extracts (TTA_{27} and TTE) on sex reversal changes in *O. niloticus* are presented in Table 4.28. The results showed that, least (53.33±11.55%) and highest (100.00±0.00%) males varied significantly across all groups of fish. The highest male percentage was recorded in fish fed control diet, MT and those fed diets that had 1.5 g/kg TTA_{27}; 2.0 g/kg TTA_{27}; 2.5 g/kg TTA_{27}; 2.0 g/kg TTE and 2.5 g/kg TTE, this was followed by the fish fed diets that contain 1.5 g/kg TTE and 1.0 g/kg TTA_{27} (93.33± 11.55%) and significantly ($p < 0.05$) least males (53.33±11.55%) were recorded in fish fed 0.0 g/kg extracts which were not different from fish fed diets with 0.5 g/kg TTE extracts. Percentage females of *O. niloticus* in this study on the hand, showed an inverse result to the male percentage where the highest females (46.67±11.55%) was recorded in fish fed diets that contain 0.0 g/kg extracts, this was not significantly different from those fed diets that contain 0.5 g/kg TTE while, least females (0.00±0.00%) were obtained in fish fed diets from 1.5 g/kg and 2.0 g/kg TTA_{27} and TTE extracts respectively which were not significantly different from those fed MT.

Table 4.28. Interaction Effects of Extract and Inclusion Levels of *T. terrestris* Extracts on Sex Reversal Changes in *O. niloticus*

Sex ratio	MT	0.0	TTA_{27} (g/kg)					TTE (g/kg)				
			0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Females (%)	0.00±0.00^{b}	46.67±11.55^{a}	13.3±11.55^{b}	6.67±11.55^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	20.0±20.0^{ab}	13.3±11.55^{b}	6.67±11.55^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Males (%)	100±0.00^{a}	53.3±11.55^{b}	86.7±11.55^{a}	93.3±11.55^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	80.00±20.0^{ab}	86.7±11.55^{a}	93.3±11.55^{a}	100±0.00^{a}	100±0.00^{a}

Means with the same superscripts along the column are not significantly different (p>0.05)

CHAPTER FIVE

DISCUSSION

5.1 Chemical and Nutrient Content Analysis of *Tribulus terrestris*

5.1.1 Phytochemical analysis of *T. terrestris*

Phytochemical screening of *T. terrestris* dried whole plant confirmed the presence of alkaloids, flavonoids, saponins, anthraquinones, terpenoids and steroids. The results of this study vary slightly from the reports of Chhatre *et al.* (2014); Usman *et al.* (2007) who recorded the presence of cardiac glycosides and tannins in their studies, while Dwivedi and Sengar (2018) reported the presence of cardiac glycosides in ethanol and water extracts of *T. terrestris*. These phytochemicals were however, absent in the current study. Ethanol, ethyl acetate and aqueous (at 27 ^{0}C) extracts of *T. terrestris* followed this same trend to reveal the presence of alkaloids, flavonoids, saponins, anthraquinones, terpenoids and steroids, this was in tandem with reports of several authors where different parts of the *T. terrestris* were analyzed using different solvents (Ammar *et al.*, 2018; Dwivedi and Sengar, 2018; Vasait, 2017; Shahid *et al.*, 2016; Abudayyak *et al.*, 2015; Pandey, 2014; Gincy *et al.*, 2014; Mathur and Sundaramoorthy, 2013; Verma *et al.*, 2009). These authors had differing reports on the presence of alkaloids as some authors reported the absence of alkaloids while others report a positive testing. In this study however, alkaloids were present in ethanol, ethyl acetate and in aqueous (at 27 ^{0}C) extracts but was absent in aqueous (at 100 ^{0}C) extract, this can be attributed to the instability of alkaloids which can be destroyed, decomposed or degraded by exposure to several factors such as moisture, air, chemicals and heat (Hossain *et al.*, 2013).

In powdered dried aerial parts of*T. terrestris*, Ammar *et al.* (2018) found volatiles, glycosides, sterols, terpenes, coumarins and traces of nitrogenous compounds among other phytochemicals whereas Vasait (2017); Pandey (2014) both found no alkaloids in the aqueous extract. Dwivedi and Sengar (2018) also concluded that chloroform, ethanol and aqueous extracts of *T. terrestris* leaves and roots had no alkaloid as well as aqueous extract of *T. terrestris* fruits while, chloroform and ethanol extracts of the fruits tested

positive for alkaloid leading to the assertion that though alkaloids are absent in *T. terrestris*, it can be found in the fruits when extracted using solvents of low polarity. The authors' study was contrary to results obtained in this study which revealed the presence of alkaloid in all the extracts except in aqueous at 100 ^{0}C. Similarly, the current study was in line with results obtained by Sharma *et al.* (2013) who inferred the presence of alkaloids in aqueous extracts of *T. terrestris* leaves and absence in methanol extracts of the fruits. Gincy *et al.* (2014) reported the absence of flavonoid, tannins, steroids and terpenoids. Similarly, using seven different solvents for extraction, while Maheswari and Rajendran (2018) reported the absence of flavonoids in all extracts, terpenoids in ethyl acetate and aqueous extracts, saponins in aqueous extract, tannins in ethanol and aqueous extracts and alkaloid in aqueous extract. The results of the current study are not in total concordance with these reports, this study revealed the presence of flavonoid, terpenoids and steroids in all *T. terrestris* extracts, however, this study is in agreement on the absence of tanins from ethanol, ethyl acetate and aqueous extracts of *T. terrestris*. This study also contrasts the reports of Pandey (2014); Maheswari and Rajendran (2018) both submitted that cardiac glycosides was present while steroids were absent in *T. terrestris* aqueous and ethanol extracts whereas, this study recorded the absence of cardiac glycosides and the presence of steroids in all *T. terrestris* extracts. Dwivedi and Sengar (2018); Maheswari and Rajendran (2018) reported the absence of saponins in *T. terrestris* ethanol and aqueous extracts, respectively these were not in agreement with the results of the current study that revealed abundant presence of saponins in all extracts of *T. terrestris*, with higher levels in ethanol and aqueous (at room temperature) extracts. This result however, corroborated results reported by Sharma *et al.* (2013). Steroidal saponin has been suggested to be responsible for increasing levels of androgens in the serum of in vivo models (Su *et al.*, 2009). Quantitatively, the alkaloid and flavonoid content of *T. terrestris* ethyl acetate extract in this study was similar to the result obtained by Maheswari and Rajendran (2018).

Tribulus terrestris aqueous (TTA_{27}) extract contained 32 mg/g alkaloid, 11 mg/g flavonoid, 83 mg/g saponin and 9 mg/g steroid while *T. terrestris* ethanol extract had 24 mg/g alkaloid, 20 mg/g flavonoid, 47 mg/g% saponin and 19 mg/g steroid. Ammar *et al.*

(2018) recorded 74 mg/g isolated saponins from the aerial parts of *T. terrestris* while flavonoids was 61 mg/g. *T. terrestris* aqueous (at 27 ^{0}C) and ethanol extracts in this study have a 7.55 times and 2.35 higher levels of saponin than flavonoid, respectively which were in contrast to findings of Shahid *et al.* (2015) who reported that flavonoids in the methanol extracts of *T. terrestris* was 1.5 times higher than saponins, however, this was true for ethyl acetate extract in this study where there was a higher level of flavonoid (28 mg/g) than saponin (1 mg/g). This suggested that, flavonoid in *T. terrestris* is more soluble in ethyl acetate than in water and ethanol, moreover, flavonoids are better extracted in solvents of lower polarity mixed with water (Do *et al.*, 2014). In addition, Chhartre *et al.* (2014) reported that saponin content of *T. terrestris* differ across different regions. Again, the extraction procedures and solvents used determines the biologically active compounds found in *T. terrestris* (Ugochukwu *et al.,* 2013; Pandey and Tripathi, 2014).

Plant bioactive compounds may provide a useful source of new medicines and pharmaceutical entities for enhancing fish production and health, as well as food safety and quality, while preserving the aquatic environment, according to evidence from the phytochemical constituents of *T. terrestris* used in this study. Intensive efforts must be made in exploiting plants, plant extracts or natural plant compounds as potential natural alternatives to synthetic steroids for enhancing fish productivity as well as the masculinization and feminization at different concentrations (Chakraborty, 2017; Chakraborty *et al.,* 2014).

5.1.2 **Proximate analysis of *T. terrestris* plant and *T. terrestris* Extracts**

Tribulus terrestris is fundamentally comprised of oligosaccharides (Assunção *et al.,* 2017; Hammoda *et al.*, 2013). Proximate composition of *T. terrestris*plant and extracts in this study revealed that *T. terrestris* whole plant had 13.59% moisture, with an ash composition of 24.32%, ether extract of 5.05%, crude fibre of 15.43%, crude protein of 17.28%, total carbohydrate of 24.34% but available carbohydrate of 8.91% and Gross energy of 150.11 Kcal/100g. These results differed from results obtained by Dastagir *et al.* (2013); Dastagir *et al.* (2014) where they analyzed *T. terrestris* as part of the family

Zygophyllaceae. These authors reported that *T. terrestris* in winter contained8.42% moisture content; 11.4% ash; 9.4% crude protein 11.3% fat content with 40.95%, 18.3% and 432.3 kcal/g for fibre content, carbohydrate and gross energy respectively. While in summer, *T. terrestris* had moisture content of 7.6%; ash content of 10.4% and crude protein and fat content of 12.5% and 7.1%, respectively with fibre content of 51.1%, carbohydrate and gross energy of 11.2% and 430.8 kcal/g, respectively.Proximate composition of *T. terrestris* in this study is in tandem with the report of Amirshekari *et al.* (2015) in which, they reported that, *T. terrestris* used in their study had 94% dry matter (6% moisture), 16.7% crude protein, 9.2% crude fibre, 7.8% ash content and 2.7% crude fat. Again, results of this study is similar to those obtained by Singh *et al.* (2010); Hassan *et al.* (2005) from analyzed *T. terrestris* in Kebbi and Sokoto States of Nigeria. This gives credence to reports that *T. terrestris* varieties differ in both phytochemical and nutrition composition across different regions.

Extracts of *T. terrestris* had varying nutritional compositions from the whole plant. Moisture content was lower in ethanol extract compared to other extracts though, it didn't differ from the moisture in aqueous (at 27 ^{0}C) extract. The moisture content of *T. terrestris* in this study was notably lower than that obtained by Singh *et al.* (2010), these authors both only analyzed *T. terrestris* leaves in contrast to the current study where whole plant was analyzed, this difference in moisture content can be attributed to the high water content in the leaves of plants, however, the reports of Dastagir *et al.* (2014; 2013) and Tholkappiyan *et al.* (2011) have mean moisture content values of 7.6%, 8.42% and 25.66% which are close to the moisture content of whole plant obtained in this study and the moisture value gotten by Hassan *et al.* (2005) is in tandem with the moisture of the extracts obtained in the current study.

Total ash content of *T. terrestris* reported in this study for whole plant (24.32%) was higher than those reported by Amirshekari *et al.* (2015), Dastagir *et al.* (2013; 2014); Adinortey *et al.* (2012) yet, the value is in tandem with those obtained by Tholkappiyan *et al.* (2011) in India and Singh *et al.* (2010) in Kebbi State, Nigeria. The ash contents of all the *T. terrestris* extracts were lower than the whole plant, the ash of the extracts was similar to the ash content of *T. terrestris* gotten (14.33g/100g or 14.33%) by Baruah *et al.*

(2017). The high ash content in this study gives an indication to the rich source of inorganic minerals element that may be present in the soil where the analyzed *T. terrestris* samples were collected.

Ether extract was lower in the whole plant of this study compared to the extracts but the all *T. terrestris* extracts had comparable ether extracts. Dastagir *et al.* (2013; 2014) reported varying values of ether extracts in different parts of *T. terrestris* during winter and summer respectively, with the fruits having the highest value of 13.4% and 9.3% followed by the stem, having 13.3% and 8.4%. The leaves and roots however, had 9.9%; 8.9% and 8.9%; 2.1% for winter and summer, respectively resulting in a mean value of 11.3% and 7.1% for the analyzed *T. terrestris* by these authors. This closely resembles the values of ether extract obtained in this study, and ether extract value reported by Hassan *et al.* (2005) was very similar to the value gotten for the whole plant in this study however, other authors have reported differing values for ash content of *T. terrestris* in their analyses; Baruah *et al.* (2017) reported 1.39%; Amirshekari *et al.* (2015) reported 2.7% and Singh *et al.* (2010) recorded 1%.

Crude protein value in the study differed between the whole plant and its extracts. The values ranged from 11.9% in ethanol extract to 17.28% in whole plant. Several authors have reported different crude protein values for different part of *T. terrestris* such as; 16.63% (Ammar *et al.*, 2018); 7.22% **(**Umadevi and Srinathrao, 2017); 14.59% (Baruah *et al.*, 2017); 16.7% (Amirshekari *et al.*, 2015); 13.1% and 12.5% (Dastagir *et al.*, 2013; 2014); 13.21% (Singh *et al.*, 2010) and 21.33% (Hassan *et al.*, 2005). These findings of crude protein values reported by these authors all supported crude protein values gotten in this study for *T. terrestris* and its extracts. Every part of *T. terrestris* predominantly have less than 20% protein content which indicate that, *T. terrestris* is not a protein source.

The crude fibre value for the whole plant (15.43%) in this research was significantly greater than the results for the extracts, indicating that *T. terrestris* is a fibrous weed. The roots have been reported to contain higher crude fibre than any other part of the plant (Dastagir *et al.*, 2013), the authors further reported 55.2%, 15.5%, 37.1% for stem, leaves

and fruits of *T. terrestris*. The result in this study is similar to reports of Hassan *et al.* (2005); Singh *et al.* (2010) both recorded 13% and 19.0% crude fibre content in Sokoto and Kebbi States of Nigeria, respectively and Adinortey *et al.* (2012) who reported 13% crude fibre value. As extracts are mostly chemical composition of a plant, it was therefore apposite that the extracts had significantly lower values than the whole plant which contained all the fibres in the plant, the fibre content in the extract were almost not detected. The high value of the whole plant in this present study also suggests that *T. terrestris* can serve as a good source of fibre.

Carbohydrate levels in *T. terrestris* was reported in Dastagir *et al.* (2014; 2013) as 11.2% and 18.5% during summer and winter respectively while, Hassan *et al.* (2005); Singh *et al.* (2010) both reported a higher value of 55.67% and 46.79%, respectively and Adinortey *et al.* (2012) stated that *T. terrestris* had 55.67% total carbohydrate. While the first two authors did not state if their values were for total carbohydrate or available carbohydrate, the current study recorded a total carbohydrate of 24.34% and available carbohydrate of 8.91%. These values differ from those previously reported, however, Baruah *et al.* (2017) stated that *T. terrestris* had a carbohydrate content of 28.18% whileDastagir *et al.* (2014; 2013) reported that in winter, the carbohydrate contents were highest in the leaves and fruits of *T. terrestris* (37.2% and 19.3%) while during summer, the roots and leaves had the highest carbohydrate content (23.5% and 10.6%). The carbohydrate content obtained in this study falls within the ranges reported by these authors. *Tribulus terristris* can be classified as a good source of energy due to its high carbohydrate content, this may be the reason why cattle and goats graze on *T. terrestris* in Northern Nigeria.

5.1.3 Proximate constituents of experimenta1 diets that contain *T. terrestris* extracts

For optimum growth, nutrient requirements of fish must be considered and the nutrient composition of the experimental diets used in this study followed the nutrient requirements for *Oniloticuss*. The proximate analysis of the experimental diet in this study showed that, the diets used in this study was isonitrogenous and isocaloric.

The moisture of the experimental diets in this study ranged from 7.26% to 7.47%, this was in line with the recommendation of Ng and Leger, (2013) who recommended that the moisture in *O. niloticuss* fry diet should be less than 10%. The ash content of the diets ranged from 6.28% to 6.31% while ether extract varied from 4.06% to 4.13%, these were within the range recommended by Ng and Leger, (2013) for the ash and crude lipid content of diets for *O. niloticuss* of less than 10g, the values of ash and ether extracts in this study were adequate for optimum growth. The ash content and ether extract content of experimental diets used in this study was lower than ash and crude fat of the diets used by Gültepe *et al.* (2014) whose ash content ranged from 9.02% to 9.95% and crude fat from 10.02% to 10.05%. The crude fiber of the diets used in this study were slightly higher than was recommended for *O. niloticuss* fry, however, Mjoun *et al.* (2010) reported that, *O. niloticuss* can tolerate higher levels of dietary fibre compared to other species, this suggested that, despite the slightly higher crude fibre used in the current study, the fibre level did not affect the growth of the fish.

Protein is a very important nutrient component of fish's diet as deficiency in protein may lead to stunted growth. The crude protein of experimental diet varied between 45.56% to 45.62%, a basal diet of 45% was formulated following the recommendation of Mjoun *et al.* (2010) who recommended that, *O. niloticuss* fry required crude protein between 30% to 56%. The crude protein level of this experimental diet was also in line with the recommendation of Ng and Leger (2013) who reported that, first feeding *O. niloticuss* larvae require between 40% and 50% crude protein while fry and fingerlings required between 30% and 40% crude protein level. The crude protein in this study is also in consonance with reports of Abdel-Tawwab (2012) who tested the effects of dietary protein on growth of *O. niloticuss* and concluded that diets of 45% produced optimum growth of *O. niloticuss*. Similarly, Nian *et al.* (2017) reported good growth performance of *O. niloticuss* fry fed diets of 45% dietary protein levels with no negative effects.

The ether extract and available carbohydrate levels of the experimental diets were also within the recommended levels for optimum growth of *O. niloticuss*. Ether extract ranged from 5.55% to 5.57% while the available carbohydrate was from 25.46% to 25.65%, respectively. Ng and Leger (2013) recommended that, the minimum lipid requirement for

O. niloticuss was 5% while Mjoun recommended a 5.2% dietary lipid for *O. niloticuss* of less than 2.5g. These authors agreed that, there is no carbohydrate level requirements for *O. niloticuss* as this fish is capable of utilizing carbohydrate as high as 40% stating that, carbohydrates in the feed of *O. niloticuss* serve as a cheap source of energy and help to improve the binding properties of pellets. Further, while recommending a carbohydrate level of more than 25% for *O. niloticuss*, Ng and Leger (2013) emphasized that, smaller fish require less carbohydrate than bigger fish.

5.1.4 **Proximate composition of fish fed diets with *Tribulus terrestris* extracts**

Oreochromis niloitcus is an important food fish in many African countries including Nigeria, this has been attested to by Olopade *et al.* (2016). The importance of *O. niloticuss* can be attributed to its nutritious flesh and quick growth and ease of culture as well as being a good source for animal protein and other mineral elements for good health. The nutrient composition of *O. niloticuss* fed diets that contain *T. terrestris* extracts in this study were not significantly variant from those fed diets with 0.0 g/kg extracts.

The moisture content of *O. niloticuss* according to FAO 2010 and USDA, 2010 should range between 78-90%. The moisture content of experimental fish in this study was higher than moisture content of fish reported by Job *et al.* (2015); Sultana *et al.* (2012), however, the result in this study was lower than reports by Kwikiriza *et al.* (2017). The result in the current research was close to moisture content of 89. 51% and 81.67% recorded by Premarathna *et al.* (2018) and Olopade *et al.* (2016), respectively. The slight increase above the upper limit of the FAO (2010) recommendation may be as a result of the small size of the analyzed samples since moisture content decreases with increase in size as reported by Job *et al.* (2015).

Ash content in this study for fish fed diets that contain both *T. terrestris* aqueous and ethanol extracts ranged between 14.0% and 14.02%. These values are similar to results obtained by several authors such as Bamba *et al.* (2014) who recorded an ash content value of 14.2% - 15.2% when they fed *Oreochromis niloitcus* diets that contain crop

residues. The result of this study also conforms with the results obtained by Abdel-Tawwab and Abbass (2016) who fed *Oreochromis niloitcus* with dry whey meal and the fish had ash content ranging from 13.9% to 14.3%. The results for ash content in the current study was in tandem with results presented by Yeganeh *et al.* (2017) who supplemented the diets of *Cichlasoma nigrofasciatum* with *T. terrestris*. These results were however contrary to results obtained by Premarathna *et al.* (2018); Jim *et al.* (2017); Olopade *et al.* (2016); Mohamed *et al.* (2016) who recorded ash values for *O. niloticuss* ranging from 0.46% to 3.33%. The flesh ether extract is controlled by the available nutrition and maturity of fish. *Oreochromis niloitcus* is an omnivore with a diet consisting of diverse food items, including algae, and zooplankton but being a white muscled fish, ether extract level are considerably lower compared to red muscled fish. According to Jim *et al.* (2017), the normal lipid content of *O. niloticuss* was 2.75%. The result of ether extract found in this study ranged from 13.39% to 14.33% which was lower than the FAO (2010) and USDA (2010) limit for crude fat of *O. niloticuss*. This result was nonetheless similar to results obtained by Tonye and Sikoki (2014) who recorded an ether extract value of 13.17% for *O. niloticuss*. Kwikiriza*et al.* (2017); Abdel-Tawwab and Abbass (2016); Bamba *et al.* (2014); Petenuci *et al.* (2008) in Sultana *et al.* (2012) all recorded crude fat content ranging from 11.7% to 18.6%. The ether extract result of *O. niloticus* in this study agreed with the conclusion of Tonye and Sikoki (2014) that, ether extract level is dependent on the flesh muscles of the fish.

Crude protein of *O. niloticus* in the current study is similar to results obtained by Kwikiriza*et al.* (2017), who reported a crude protein of 64.50% to 69.30% for different strains of *O. niloticus* collected from different locations in Uganda. Abdel-Tawwab and Abbass (2016) on other hand, observed crude protein ranging from 65.8% to 66.5% for *O. niloticus* fed dried whey meal. Bamba *et al.* (2014) obtained between 66.3% and 66.5% crude protein for *O. niloticus* fed with diets that contain crop residues.

5.2 Growth performance and survival of*O. niloticuss* fed diets with *T. terrestris* Aqueous (at 27 ^{0}C) and Ethanol extracts

Growth of fish is one of the factors that can lead to improved productivity in aquaculture especially in Tilapia production, therefore, the growth and survival of *O. niloticus* is important in increasing and sustaining aquaculture development particularly in Nigeria

and other developing countries (Waite *et al.*, 2014). Citarasu (2010) reported that phytochemicals enhance various activities like growth, feed consumption, as well as act as tonic in immune-stimulation while promoting antimicrobial properties of fish.

The growth response and survival of *O. niloticus* in the current study revealed that, there was similar growth performance and survival between *O. niloticus* fed diets that contain *T. terrestris* aqueous extracts and those fed diets that contain *T. terrestris* ethanol extract. This result is in accordance with the results obtained by Chakraborty (2017) who reported that, solvent for extraction had no visible effects on growth and survival of *O. niloticus* fed diets with *B. alba* leaves and *T. terrestris* seeds extracts. Despite the similarity in the growth performance produced by these extracts, numerically, *T. terrestris* aqueous extracts gave a higher growth performance which contracted the findings of Chakraborty (2017) who found that ethanol extracts produced numerically better growth responses. Again, in line with the study of Chakraborty (2017), this study found out that, growth varied among the inclusion levels of *T. terrestris* extracts, however, inclusion level had no effects on the survival of the fish, this supported the conclusion of Chakraborty (2017); Ghosal *et al.* (2015), that, addition of *T. terrestris* extracts has no negative effects on *O. niloticus*. Similarly, the results in this study show that, like the reports of the above authors, there were significant interaction effects of solvent used for extraction and inclusion level of *T. terrestris* on the growth and survival of *O. niloticus*.

There was increase in mean weight gain of experimental fish fed diets with increasing *T. terrestris* extracts in the current study, this supported the findings of Kavitha and Subramanian (2011); Omitoyin *et al.* (2013) on *Peocilia latipinna* and *O. niloticus*, respectively. These authors reported that fish had better growth with increase in the concentration of *T. terrestris* in culture water and feed, respectively. When fed diets containing *T. terrestris* extracts ranging from 2.0g/kg, the fish in this study gained weight in a similar way as fish fed diets with 17-α-methyltestosterone. This suggested that in terms of growth, *T. terrestris* extracts when added to fish diets produce similar growth responses as MT treated fish with and does not inhibit growth of fish. Chakraborty (2017), Omitoyin *et al.* (2013); Turan and Cek (2007) further observed that *T. terrestris* does not impede the growth of *O. niloticus* and *Clarias gariepinus* respectively. Yeganeh

et al. (2017) reported a 3.23 g weight gain after feeding convict cichlids *Cichlasoma nigrofasciatum* 1.0 g/kg of *T. terrestris* for 45 days which was similar to the result of the present study. Babajhajiani *et al*. (2018) discovered that there were no significant changes in the growth of *C. nigrofasciatum* fed diets with *Urtica diioca* roots and *T. terrestris* whole plant extracts, contrary to the findings of the current investigation and the conclusions of the aforementioned authors. They further reported a decline in growth with increase in these extracts leading them to conclude that, this decline may have been as a result of extract type and related compounds.

Specific growth rate (SGR) observed for fish fed diets with *T. terrestris* extracts in this study was similar to results obtained by Tonye and Sikoki (2014). *Oreochromis niliotcus* fed diets with *T. terrestris* extracts exhibited a corresponding increase in specific growth rate with higher concentration of *T. terrestris* in the diet. This result agreed with results in the studies reported by Babahajiani*et al.* (2018); Yeganeh *et al.* (2017); Shamsuddin *et al.* (2012). Karpagam and Krishnaveni (2014) also, reported that *Oreochromis mossambicus* fed *Moringa oleifera* supplemented feed exhibited similar specific growth rate with the current study, they however noted that, SGR started declining after the first 15 days of treatment**.**The SGR result in this study was contrary to results obtained by Madalla *et al.* (2016) when *Oreochromis niliotcus* showed a negative growth correlation with increase in cassava leaf meal however, they suggested that this may have been as a result of poor digestibility of the cassava leaf meal by the fish. Many other authors have reported the SGR of *O. niloticus* cultured in different facilities to fall within the range of the results obtained in the current study (Alhassan *et al.*, 2018; Ahmed *et al.*, 2013) while Shamsuddin *et al.* (2012) reported that the SGR of fish fed supplementary diet was between 3%/d and 4%/day which was similar to the result obtained in the present study. The SGR of O. *niloticus* fed diets with *T. terrestris* extracts were similar to those fed MT treated diet suggesting that, *T. terrestris* can act comparatively with MT as an androgeneous feed additive for O. *niloticus*. This assertion is drawn from the conclusions of Odin *et al.* (2009) and Mubarik (2011), who recorded increased specific

growth rates with increase androgen levels in *O. niloticus* using testes of boa, cattle and hog and in *Cyprinus carpio* respectively.

The survival of fish in the current study is high and similar to those reported by Chakraborty (2017); Ghosal *et al.* (2016); Kavitha and Subramanian (2011) when they fed fish with *T. terrestris*supplemented feed. Chakraborty (2017) reported an increasing survival with increase in *T. terrestris* and recorded as high as 97.5% survival in fish fed diets with 5 g/kg in his study.On the contrary, Janalizadeh *et al.* (2018) reported that, fighter fish *Betta splendens* exhibited a decreasing survival when fed with *T. terrestris* treated enriched artemia. Bamba *et al.* (2008) recorded 75 to 94 % survival after 90-day trial for Nile tilapia fed diets that contain composite mixture of cocoa bean shell and coconut oil cake. *Cichlasoma nigrofasciatum*fed with *T. terrestris* showed 100% survival (Yeganeh *et al.*, 2017) while *O. niloticus* fed *Basella alba* and *T. terrestris* had 94.96% survival (Ghosal*et al.*, 2015; 2016) giving evidence that *T. terrestris* can produce a 100% survival and not cause harm that can lead to death of the fish.Agreeing with Alok *et al.* (2018) that using commercial feed, the survival of 76.5% for *O. niloticus* is in good range, this study confirms that *T. terrestris* extract was not harmful to *O. niloticus*.

*Oreochromis niloticus*fed diets with *T. terrestris* extracts demonstrated a similar growth and survival as those fed with MT treated feed concurring with the conclusion of Omitoyin *et al.* (2013) that *T. terrestris* have anabolic effects, since *O. niloticus* fed diets with varying inclusion levels of *T. terrestris* extracts along with the control group showed better growth performance compared to fish fed diet with 0.0 g/kg*T. terrestris* extract. Many researchers have also reported their findings on effects of different plant extracts on growth improvement of fish lending support to the findings in this current study. Manaf *et al.* (2016) recorded improved growth of fish fed diets supplemented with 3.5 g/kg methanol extracts of *Vitex trifolia, Strobilanthes crispus* and *Aloe vera* for 60 days, noting that, fish diets fed these extracts had better growth compared to those fed diets without the mixture. *Allium sativum* and *Foeniculum vulgare* iols used as additives individually and in combination with a bacterium in fish diet resulted in higher weight gain and specific growth rate of the fish (Hassan and Soltan, 2016). Furthermore, Kareem *et al.* (2016) reported improved growth of *O. niloticus* fed feed supplemented with

Azadirachta indica, *Carica papaya*, *Cinnamomum camphora* and *Euphorbia hirta* extracts, adding that, only the fish fed feed with *A. indica* showed similar growth as the fish fed diets with no extracts. Additionally, Adenigba *et al.* (2017) and Nian *et al.* (2017) fed *C. gariepinus* and *O. niloticus* with pollens of *Pinus tabulaeformis*, respectively and concluded that, the pollens had a positive effect on the growth of these fish species. Furthermore, *Phoenix dactylifera* seed extract were tested for their growth effects on *Cyprinus carpio*, it was concluded that, this extract can improve the growth of *C. carpio* at 0.5% body weight or by replacing 10% plant sources with *P. dactylifera* in *C. carpio* feed (Mohammadi *et al.*, 2018; Kamali-Sanzigh *et al.*, 2018).*Aloe vera* polysaccharides at between 1.76% to 1.79% per kilogram also improved the growth of *C. gariepinus* (Gabriel *et al.*, 2019).

5.3 Water Quality Parameters of Fish Culture Tanks

Abiotic factors particularly water quality are important for the survival and growth of *O. niloticus* under culture conditions. Consequently, it is believed that the suitable range of water quality parameters ensure the better management of aquatic organisms and environment (Begum *et al.*, 2014). Water quality parameters of experimental set up are in conformity with the requirements for adequate growth of *O. niloticus* as reported by El-Sherif and El-Feky (2009), Soto-Zarazúa *et al.* (2009).

Water temperature, being one of the most important water quality parameters that have an effect on food intake and metabolism, growth, reproduction as well as other biological activities of fish (Mjoun, 2010) played an important role in the growth and survival of *O. niloticus* in this study. Contrary to the results obtained in this study, Begum *et al.* (2014) recorded temperature varying from 30 ^{0}C to 34.5 ^{0}C while quoting that the optimum temperature for suitable fish culture was between 28 ^{0}C to 35 ^{0}C. Water temperature, in this study was in conformity with the result recorded by Dereje *et al.* (2015); Choudhary and Sharma (2018), though, there were higher values recorded in the current study compared to the work of Zebib and Teame (2017), these authors agree that a water temperature between 20 ^{0}C and 30 ^{0}C was optimum for Tilapia growth. Furthermore, the water temperature recorded in this study was in line with the submission of Cline (2012)

who stated that, optimum range for Tilapia culture is between 23.89 ^{0}C to 34.4 ^{0}C and Mjoun (2010) who also, stated that optimum temperature for good growth is 22 ^{0}C to 29 ^{0}C adding that, tilapia rarely survive temperature lower than 10 ^{0}C.

The pH in the current study was in congruence with the result of Cadini *et al.* (2011); Workagegn *et al.* (2014), these authors recorded a pH varying from 7.32 to 7.84 when they treated *O. niloticus* with different types of diet from variety of ingredients. On the contrary, Makori *et al.* (2017) recorded pH levels lower than obtained in the current study and reported that, this consequently affected the growth of *O. niloticus* in their study. In addition to the results of this study conforming to those obtained by Bilale and Teklie (2017), this study agrees with the authors that *O. niloticus* can survive a wide range of pH (Ovie *et al.*, 2011) with an optimum range of 6.5 to 9. According to Begum *et al.* (2014),maintaining good levels of dissolved oxygen in water is important for fish production. Dissolved oxygen has a direct impact on fish's feed intake and metabolism as well as its disease resistance and growth. Many authors have reported that, it is important to maintain dissolved oxygen above 3.0 mg/L for fish culture (Ekubo and Abowei, 2011; Cline, 2012; Bhatnagar and Devi, 2013), The dissolved oxygen in the current study was within the normal range for *O. niloticus* culture which in tandem with results of the works of Makori *et al.* (2017). However, dissolved oxygen in the current study was considerably lower than was recorded by Omitoyin *et al.* (2013) though the authors reported using aerators for their study which may have contributed immensely to the higher dissolved oxygen level recorded in their study compared to f the current study. It is worthy of note that, the absence of aerators in the current study did not debilitate the survival or growth of fish in the present study. This further suggested that, *T. terrestris* in water does not affect the dissolved oxygen level and is not toxic to fish.

Nitrogenous compounds in fish culture water is inevitable, while ammonia is due to protein metabolism and bacterial decay of organic matter, nitrite is the intermediate by-product of the transformation of ammonia to nitrate by nitrifying bacteria. Ammonia as low as 0.6 mg/L have been reported to be toxic to fish and other aquatic organism on short term exposure (Begum *et al.*, 2014; Bhatnagar and Devi, 2013) while Mjoun (2010) stated that ammonia levels between 2.5 mg/L and 7.1 mg/L were toxic to *O. niloticus*.

Ammonia and nitrite results in this study was in tandem with the acceptable levels for fish according to Bhatnagar and Singh (2010), this was also in line with the result of Makori *et al.* (2017); Begum *et al.* (2014); Cline (2012).

5.4 Haematology and Serum Biochemistry of *O. niloticus*Fed Diets containing *T. terrestris* Extracts

It is well known that haematological characteristics are used as indicators for diagnosing diseases and stress-linked conditions, as well as for evaluating feed additive (Fagbenro *et al.*, 2013). Haematolical changes are very vital in ascertaining fish well being due to stress and other related issues. Most times, this haematology investigations are done for diagnostic purposes while examining the influence of feed additives on the stress level of fish. Blood analysis is an important tool for measuring the health conditions of farm-raised fish and calculating the impact of diets and other stressors on their health. Changes in fish haematology in reaction to stressors are signs of the fish's stressed stage, providing valuable information for preventing any unfavorable conditions that might damage the fish's wellbeing.

5.4.1 Haematological changes in *O. niloticus* fed diets with *T. terrestris* extracts

Haematological changes are characteristically used to evaluate physiological, environmental and pathological indicators in fishes (Akinrotimi *et al.*, 2012; Gabriel *et al.*, 2011; Satheeshkumar *et al.*, 2011), this has led to the examination of different haematological indices such as hemoglobin concentration (Hb), hematocrit (Hct), platelets, white blood cell (WBC) along with other signs of anaemic conditions and differentials derived from these indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocytes, monocytes and eosinophils) to ascertained the health conditions of fish species (Gabriel, 2019). Accordingly, Jimoh *et al.* (2015) asserted that haematological and biochemical indices are good indicators of the health status of farmed fish hence, the need to investigate the hematological changes in *O. niloticus* fed diets that contain varying levels of *T. terrestris* extracts to ascertain the health status of fish fed these extracts.

Results in this study showed that solvent used for extraction of *T. terrestris* produced significant changes in some haematological parameters of fish fed diets with different

extracts. Haemoglobin concentration, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and lymphocytes were significantly lower in fish fed diets with *T. terrestris* aqueous extracts compared to those fed diets with *T. terrestris* ethanol extracts while monocytes were higher in fish fed *T. terrestris* aqueous extracts. Similarly, there were significant effects on these haematological parameters of fish fed different inclusion levels of the *T. terrestris* extracts, these different inclusion levels elicited different haematoligical responses from the fish. The results of Hb concentrations in this study are comparable to the results obtained by Sayed and Moneeb (2015) who studied the haematological and biochemical indices of MT treated Nile Tilapia and reported haemoglobin values between 7.6 (g/dL) to 8.42 (g/dL). Haeamoglobins are the oxygen carrying cells of the body. The decrease in Hb concentrations with increase in *T. terrestris* extract concentration indicates that, though *T. terrestris* extracts showed evidence of reducing the oxygen carrying capacity of fish fed these diets, it was evident that, Hb concentrations were higher in fish fed *T. terrestris* extracts compared to those with no extracts. This is in line with the assertions of Moradikor *et al.* (2013) where they reported that *T. terrestris* was not harmful to humans and animals. Hemalatha and Hari (2015) in their report observed that male albino rats treatedwith saponin fraction of *T. terrestris* extract showed no significant difference in their Red Blood Cell (RBC) counts and Hb levels compared to the control group. Meanwhile, in the current study, *O. niloticus* fed with diets with *T. terrestris* extracts had comparative Hb levels with those fed with MT treated diet. Moreover, the survival of these treated fish indicated that, with increase in the levels of *T. terrestris*, there was higher survival of the fish. Fish fed *T. terrestris* treated diets exhibited erythrocyte swelling which were evident in the slight increase in MCV values of fish fed diets with *T. terrestris* extracts compared to those fed 0.0 g/kg*T. terrestris* extract, which is similar to the observations of Sayed and Moneeb (2015); Gültepe*et al.* (2014). These authors reported no considerable effect of *T. terrestris* extracts in the haematological profile of *O. niloticus*. Similarly, the results of this work follows the trend obtained by (Obaroh *et al.*, 2014) when they fed *O. niloticus* with diets that contain of *Magnifera indica* leaf extracts and reported an increase in the Hb, PCV, RBC and WBC counts in male and female *O. niloticus* fed diets with the plant

extract in comparison to those fed diets without the extracts. According to Witeska (2013), erythrocyte count depends on fish activity, water temperature, other environmental factors as well as age, sex, nutrition and reproductive status.

The measure of the oxygen carrying capacity of blood is expressed as the hematocrit (Hct), this therefore, is the percent of blood volume filled with erythrocytes compared to total blood volume. In the present study, there was an almost uniform Hct across all fish fed *T. terrestris* extracts diets ranging from 25.13 g/dL to 25.73 g/dL. These values are similar to results obtained by Sayed and Moneeb (2015) and Dal'bo *et al.* (2015). However, Gabriel *et al.* (2011) recorded a considerably lower HCT/PCV values for *O. niloticus* from the wild held in captivity. The Hct result in this study followed the trend reported by Kefi *et al.* (2013) who posited that, this change may be as a result of the androgen administration which suggests that *T. terristris* can act as an androgen agent. Though, lower Hb levels may inhibit fish from certain process such as reduced metabolism, fish in this study exhibited higher Hb values compared to results obtained in the experiment carried out by Gültepe *et al.* (2014). The decrease in Hb and consequently, HCT with increase in *T. terrestris* extracts in this study was similar to the observations Mekkawy *et al.,* (2013); Mekkawy *et al.,* (2010); Adedeji *et al.,* (2009). When they exposed fish to substance such as atrazine, cadmium and diazinon. Moreover, the levels of Hct of fish in the current study fell within the normal range 20% to 30% Hct for fish which was reported by Satheeshkumar *et al.* (2011) who further stated that, Hct of fish are rarely ever higher than 50%.

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) significantly increased with increasing *T. terrestris* extract in the blood of fish fed diets containing *T. terrestris* extracts. These results follow the same pattern observed by Sepperumal and Saminathan (2013) in Diethyl phthalate treated fishes; Bohlouli and Sadeghi (2016) in *Oncorhynchus mykiss* fingerlings supplemented with dietary *Ferulago angulata*.

Results in the present study corroborated the reports of Dal'bo *et al.* (2015) who reported that platelets are the most abundant blood cells after RBC. There was a gradual decrease

in the platelets in this study which was in contrast to the report of Diyaware *et al.* (2013) who reported an increase in platelets in hybrid clariid fish. This contrast can be attributed to the differences in fish species studied. This study was however in tandem with the study of Obaroh*et al.* (2014), who treated *O. niloticus* with crude *Magnifera indica* leaf extracts. Platelets, known as the clotting agents of the blood were not significantly different in all fish fed *T. terrestris* extracts based diets from those fed 0.0g/kg*T. terrestris* extract. Despite the decreasing platelets in fish fed *T. terrestris* ethanol extracts, *O. niloticus* can easily heal from injuries.

White Blood Cells (WBC) levels in this study was almost uniform across all treatments of fish fed diets with *T. terrestris* extracts, these were also comparable to WBC of fish fed control diet, MT and those fed diets with 0.0 g/kg*T. terrestris* extract. In the present study, lymphocytes were found to be the most common leukocytes which represent the functionality of the cell immunity of fish this was followed by monocytes and eosinophils. While Lymphocytes count did not follow the same pattern as WBC, Monocytes and Eosinophils followed the same trend as WBC. This trend is similar to the observations of Uluköy *et al.* (2018); Dal'bo *et al.* (2015); Hrubec and Smith (2010). A high number of WBC would indicate an immune response of the fish to disease, stress or toxicant. In the present study, there was a uniform WBC count indicating that the fish in all groups have no significant response to *T. terrestris* extracts as a health threat to their immune systems. This submission is however, not in tandem with the results of Bohlouli and Sadeghi (2016) who stated that WBC and its differentials increased in *Oncorhynchus mykiss* fed *Ferulago angulata* supplemented diets. Manish *et al.* (2009) reported a lower WBC levels when Swiss albino mice were injected with *T. terrestris* root extract after six hours but increased to normal levels after 24 hours, while Ismaiel *et al.* (2017) reported a decreased WBC in *T. terrestris* ethanol and ethyl acetate extracts treated rabbits supporting the claim that, *T. terrestris* does not elicit an immune response. The current study revealed a slight decrease in lymphocytes percentage across all treatments though these results were not significantly different. The increasing Monocytes levels with increase *T. terrestris* extract agrees with the observations of Baba *et al.* (2014) when *Sparus aurata* was fed *Muscari comosum* plant extract. Adi *et al.* (2017) corroborated the

submission of Akinrotimi and Gabriel (2012) where these authors reported that increase in WBC and monocytes showed an immune response to fight stressor in fish. The results of the present study however shows that *T. terrestris* extract may probably not necessarily stimulate immune responses of *O. niloticus*.

This is in line with the findings of Gültepe *et al.* (2014) who observed that the hematological indices of *O. niloticus* were not affected by *T. terrestris*.

5.4.2 Serum biochemistry of *O. niloticus* fed diets with *T. terrestris* extracts

The results in this study showed that there was no significant effect of extraction on the serum biochemistry of fish fed diets with *T. terrestris* extracts. Fish fed diets that contain *T. terrestris* aqueous extracts had slightly higher serum biochemistry compare to fish fed diets with *T. terrestris* ethanol extracts except seruml2 cholesterol which was lower. There was an inclusion level dependent response in Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) Alkaline Phosphate (ALP) and serum cholesterol levels across all groups of fish fed diets with *T. terrestris* extracts. However, albumin, globulin, glucose and total protein levels were significantly different in fish fed diets with the different inclusion levels of *T. terrestris* extracts.

Elevated levels of Aspartate aminotransferase, Alanine aminotransferase and Alkaline Phosphate in fish have been reported to be as a result of hepatotoxicity of liver cells (Joseph and Raj, 2011) but the evident decrease in these serum enzymes in fish fed diets with increasing *T. terrestris* extracts compared to those without *T. terrestris* extract which is an indication that, *T. terrestris* extracts did not affect the livers of the tested *O. niloticus* as Aminotransferase are known to be the bio-indicators of liver damage. The results of the current study contradicted the results of Fagbuaro *et al.* (2016) who investigated the biochemical composition of *O. niloticus*. Oner *et al.* (2009) reported a decreasing of ALT when *O. niloticus* were exposed to heavy metals, similar trend was observed in this study. Significant increase in AST and ALT were reported by Younis *et al.* (2012) which suggested hepatic damage due to zinc accumulation in *O. niloticus*. Edori *et al.* (2013) also noted that elevated AST, ALT and ALP were signs of damaged

liver caused by necrosis of cells. This study showed a decrease in these serum enzymes indicating that, the livers of the treated fish were not damaged. Albumin and Globulin levels in the current study reveal a uniform trend where fish fed *T. terrestris* extracts based diets have similar levels of albumin and globulin compared with those fed without *T. terrestris* based diets and those fed with the control diet. This trend is in contrast to the result obtained by Adewoye (2010) who treated *C. gariepinus* with *Trephosia vogelii* extract and reported that, the increase in *T. vogelii* extract and consequent decrease in albumin and globulin of *C. gariepinus* may have been as a result of kidney clearance and liver malfunction. However, in this study, the serum enzymes indicating liver function have alluded to the proper functioning of the livers of treated fish. This is in line with the observations of Lakshmi *et al.* (2011) that *T. terrestris* has a protective effect on liver and kidneys in cadmium intoxicated rats. Furthermore, the results for albumin and globulin in the present study are within the range of the results obtained by Gültepe *et al.* (2014) who concluded that, *T. terrestris* had no effect on the albumin levels of *O. niloticus* fed *T. terrestris* supplemented diets, even though a higher globulin level in fish fed the lowest. *T. terrestris* was reported in their study.

Cholesterol, a chemical compound, is a combination of lipid and steroid which are building blocks for cell membranes and for sex hormones like estrogen and testosterone (Sayed and Moneeb, 2015). Even though *T. terrestris* is acclaimed to be a testosterone booster, in this study, the results showed a lower level of cholesterol in *O. niloticus* fed *T. terrestris* extracts based diets compared to those fed with control diet and MT. This is not surprising as Sayed and Moneeb (2015) reported a significantly higher cholesterol levels in *O. niloticus* fed with MT based diet. Tagsin *et al.* (2010) and Fagbuaro *et al.* (2016) further reported that testosterone induced rats and male *O. niloticus* respectively has higher cholesterol levels compared to the females from the same environment. The present study however indicated a significantly lower cholesterol levels in fish fed diets with *T. terrestris* aqueous and ethanol extracts compared to the serum cholesterol of fish fed without *T. terrestris* extract. These results is in line with the claims that, *T. terrestris* extracts are capable of lowering blood cholesterol in humans, rats, mice and poultry (Penkov and Nikolova 2016; Grigorova *et al.*, 2014; Grigorova *et al.*, 2008). Chhatre *et*

al. (2014) also reported several evidences of *T. terrestris*' blood cholesterol lowering properties. Amirshekari *et al.* (2015) and Grigorova *et al.* (2009) suggested that phytochemicals in *T. terrestris* block the enzymes that play important roles in cholesterol synthesis, this is possibly why *O. niloticus* fed with *T. terrestris* extracts based dietsin this study had lower cholesterol levels. Glucose is a reliable indicator of environmental stress in fish (Al-Asgah *et al.*, 2015) where a depleted level may be due to utilization of energy generated by the fish (Adewoye, 2010). However, glucose level in this study was almost uniform indicating no change in the level of glucose of fish fed with diets with *T. terrestris* extracts leading to the conclusion that, *T. terrestris* did not produce a stress response in the fish. Also, the glucose levels in this study are similar to result obtained by Antache *et al.* (2014) who recorded a glucose level ranging between 89.18 mg/dL and 99.71 mg/dL in *O. niloticus*. It also falls within the reference range of 22.7mg/dL to 107mg/dL for *O. niloticus* as reported by Hamid *et al.* (2013). Ghanbari *et al.* (2016) observed a significantly lower serum glucose in diabetic rats treated with *T. terrestris* while Samani *et al.* (2016) also reported that *T. terrestris* reduced the blood sugar of diabetic women compared to the placebo. These are contrary to the results obtained by of Gültepe *et al.* (2014) in *O. niloticus* fish where there was an increase in glucose level with increase in *T. terrestris* level. The lowered serum glucose, according to Ghanbari *et al.* (2016) may be due to the antioxidant effect of *T. terrestris* extract.

The current study revealed an increase total protein in *O. niloticus* fed diets with *T. terrestris* extracts compared to those fed 0.0 g/kg*T. terrestris* extract. This result is in tandem with the results of Gültepe *et al.* (2014). The authors reported that, *T. terrestris* had no significant effect on the total protein of *O. niloticus* even though, there were relatively higher total protein values in treated fish. Increased concentrations of total proteins can be caused by structural liver alterations reducing aminotransferase activity (Francesco *et al.*, 2012) while the increase in protein could as well indicate a slight dehydration. Total protein result in this study further confirm the results of Manikandaselvi *et al.* (2013) who reported that there was no significant difference in the total protein of rats when the hepatoprotective effect of *T. terrestris* was tested. Similarly,

the total protein levels in the current study closely resembles the reportds of Sayed and Moneeb (2015).

5.5 Sex Reversal Changes in *O. niloticus* Larvae Fed Diets with *T. terrestris*Extracts

Many researches have documented and reported sex changes in different species of fish using plant extracts. These studies have demonstrated that palnt extract can influence sex differentiation in teleosts.

5.5.1 Sex ratio of *O. niloticus* fed diets with *T. terrestris* extracts

Several researchers have used herbal means or plant extracts to induce sex reversal in fish evident in the use of genistein from toasted soybean flour on *C. gariepinus* (Ahmed *et al.*, 2015); *Basella alba* on *O. niloticus* (Ghosal and Chakraborty, 2014a); *Aloe vera* on GIFT fry (Gabriel *et al.*, 2017); *Glycyrrhiza glabra* on *Poecilia reticulata* (Turan, 2017); *Mucuna pruriens* seeds and *Asparagus racemosus* roots on *O. niloticus* (Mukherjee *et al.*, 2018) among others. All these researches were against the back drop of phytochemicals being relatively safer for the environment and final consumers of sex reversed fish (Gabriel *et al.*, 2015b). *Tribulus terrestris* have been tested over time to sex reverse fish such as *Poecilia latipinna*, *P. reticulata*, *Cichlasoma nigrofasciatum,Clarias gariepinus* and *O. niloticus* (Kavitha and Subramanian, 2011; Kavitha *et al.*, 2012; Omitoyin *et al.*, 2013), however, these investigations did not use of molecular markers to ascertain the sex reversal changes in these fishes.

Ghosal *et al.* (2015), who examined the efficacy of using *B. alba* leaves and *T. terrestris* seed extracts for monosex production of *O. niloticus* reported that, aqueous extracts produced significantly lower male percentage compared to the males produced by ethanol and methanol extracts, stating that, extraction of both plant materials with ethanol produced the highest male percentage. These authors also reported that, based on concentration of plant material, the highest males were produced in 1 g/kg category of inclusion and least in 0.5 g/kg category but *T. terrestris* extract at 1.5 g/kg gave the highest male percentage. Ghosal *et al.* (2015) submitted that, there were no significant interaction effects of solvent and concentration as well as solvent and plant material on male percentage, however noted that there was significant interaction effect of solvent, plant material and concentration where *T. terrestris* ethanol extract produced the highest

males (88.9%) at 1.5 g/kg while *T. terrestris* aqueous extract had 80.8% at the same concentration.

Chakraborty (2017) reported similar results as Ghosal *et al.* (2015), however, in line with the results of this study, the author reported that, there was a higher male percentage produced from feed fish with 2.0 g/kg *T. terrestris* seed ethanol extract however, a decline in male percentage was recorded after this concentration, this was contrary to the result of this study which recorded similar male percentage as 2.0 g/kg at 2.5 g/kg.

The current study revealed an increase in the number of males with higher concentration of *T. terrestris* extract irrespective of the solvent used for extraction. This result is in tandem with the results obtained from an increased concentration of *M. pruriens* by Mukherjee *et al.* (2018) though, they reported that, this increase was only relative to methanol extracts. Kavitha and Subramanian, (2011); Kavitha *et al.*, (2012); Omitoyin *et al.*, (2013); Ghosal *et al.*, (2015; 2016) have also reported the percentage increase in male fish with increase in *T. terrestris* extracts in their various researches however, Janalizadeh *et al.* (2019; 2018) reported a contrary observation where the male percentage stared declining with increase in *T. terrestris* extract after an initial increase which was similar to the result of Ghosal *et al.* (2015; 2016) with *B. alba*.

The present study revealed that the highest percentage of male was recorded in fish fed diets with *T. terrestris* aqeuous extract, this result is in contrast to the results of Ghosal *et al.* (2015; 2016) where they reported that, the highest male percentage was recorded in fish fed diets with *T. terrestris* ethanol extract, however, these two studies have similar male percentage of fish fed diets with *T. terrestris* extracts. The explanation for this disparity may be due to the amount of steroidal saponin found in *T. terrestris* aqueous extract, which is thought to be responsible for higher testosterone (Singh *et al.*, 2012) levels in *T. terrestris* aqueous extract in the current sample, resulting in a higher number of males in fish fed *T. terrestris* aqueous extract diets than those fed *T. terrestris* ethanol extract diets.

Similar to results in this research was the results of Kavitha and Subramanian (2011) who recorded 97% males after immersing *Poecilia latipinna* fry for 60 days in *T. terrestris*

ethanol extract. Similarly, the result in this research agrees with the findings of Mukherjee *et al.* (2018) when the researchers treated *O. niloticus* with *M. pruriens*, theyreported 94% males in the ethanol extract treatment at 0.2 g/kg, while Ghosal *et al.* (2015; 2016) on the other hand, recorded a 91% males in *T. terrestris* ethanol extract treated fish at 2.0 g/kg. Having noted that the percentage of male increases with increase in *T. terrestris*, it is safe to assume that, with further increase, their result would be totally in congruence with the present study in line with the reports of Kavitha *et al.* (2012) and Omitoyin *et al.* (2013).

CHAPTER SIX

CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

7.1 Conclusion

The current study revealed that, *Tribulus terrestris* extracts have the properties to improve growth and survival of *Oreochromis niloticuss* without compromising the health status of fish treated with diets with *T. terrestris* extracts. Total sex reversal was induced in fish treated with *Tribulus terrestris* aqueous extracts from 1.5 g/kg and 2.0 g/kg *Tribulus terrestris* ethanol extracts as well as 17-α-methyltestosterone. It can therefore be concluded that even in small doses, *T. terrestris* extracts can be used as a growth enhancement agent and from 1.5 g/kg, it can be used to induce sex reversal in *Oreochromis niloticuss* larvae in place of synthetic sex reversal hormone without compromising the health of the fish. *Tribulus terrestris* having been used successfully in several other researches to promote growth and health of several species as well as induce sex reversal, it can be used successfully as a natural alternative to synthetic hormones for sex reversal in fish culture in Nigeria.

7.2 Recommendation

Further research should be carried out on the histopathological analysis of the gonads and other organs of the fish such as the liver, stomach and brain of the fish so as to ascertain the effect of *T. terrestris* on the gonads.

A hormonal assay of the tested fish is also important to ascertain the claim that *T. terrestris* increases the level of testosterone in the fish.

Toxicology test should also be carried out to verify the levels of *T. terrestris* present in the fish after the period of withdrawal.

Rearing fish to table size is also necessary to ascertain that, there would be no sex inversion after initial reversal as well as test the steroidal levels in fish, culture water and sediments to determine the biodegradability and safety of *T. terrestris*.

7.3 **Contribution to knowledge**

This research has contributed to already existing body of knowledge in the following;

- It has provided quantitative data of the phytochemical constituents of *Tribulus terrestris* extracts
- It has provided information on the most efficient solvents for the extraction of *T. terrestris* that can effectively induce sex changes in *O. niloticus*
- Baseline data of the haematological and biochemical changes in *O. niloticus* fed with *T. terrestris* extracts based diets was provided
- It has also provided information on the concentration of *T. terrestris* extracts in fish diet best suited to cause sex reversal in *O. niloticus*
- It has provided the optimum concentration of *T. terrestris* extract to produce maximum growth.

References

Abadjieva, D. and Kistanova, E. 2016. *Tribulus terrestris* alters the Expression of Growth Differentiation Factor 9 and Bone Morphogenetic Protein 15 in Rabbit Ovaries of Mothers and F1 Female Offspring.*PLoS ONE* 11.2: https://doi.org/10.1371/journal.pone.0150400

Abdel-Tawwab, M. and Abbass, F. E. 2016. Dry whey meal as a protein source in practical diets for Nile tilapia, *Oreochromis niloticus* fingerlings. *Journal of Applied Aquaculture* 28.4: 276 – 284.

Abdel-Tawwab, M. 2012. Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticuss* (L.). *International Aquatic Research* 4.3: 13 p.

Abdel-Tawwab, M., Ahmad, M. H., Seden, M. E. A. and Sakr, S. F. M. 2010. Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticuss* (L.), against *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society* 41: 203–213

Abudayyak, M., Jannuzzi, A. T. zhan, G. O and Alpertunga, B. 2015. Investigation on the toxic potential of *Tribulus terrestris in vitro*. *Pharmaceutical Biology* 53.4: 469 – 476.

Acar, Ü., Kesbic, O. S., Yilmaz, S., Gültepe, N. and Türker, A. 2015. Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against *Streptococccus iniae*. *Aquaculture* 437: 282 – 286.

Addisu, S. and Assef, A. 2016. Role of Plant Containing Saponin on Livestock Production; A Review. *Advances in Biological Research* 10.5 309 – 314.

Adedeji, O. B. and Okocha, R. C. 2011. Constraint to aquaculture development in Nigeria and way forward. *Journal of Applied Sciences Research* 7.7: 1133 – 1140.

Adedeji, O. B., Adeyemo O. K. and Agbede, S. A. 2009. Effects of diazinon on blood parameters in the African catfish (*C. gariepinus*). *African Journal of Biotechnology* 8.16: 3940 – 3946.

Adenigba, I., Tumbokon, B. L. M. and Serrano, A. E. 2017. Androgenic and Anabolic Effects of *Pinus tabulaeformis* Carr. Pollen in *Clarias gariepinus The Israeli Journal of Aquaculture - Bamidgeh, IJA_69.1388,* 8 p

Adewoye S. O. 2010. Haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract. *Advances in Applied Science Research* 1.1: 74 – 79.

Adhikari, P. Pandey, A. Agnihotri, V. Pande, V. 2018. Selection of solvent and extraction method for determination of antimicrobial potential of *Taxus wallichiana* Zucc. *Research in Pharmacy* 8: 9 p.

Adi, G., Akinrotimi O. A.and Nwokoma, U. K. 2017. Haematological changes in African catfish (*Clarias gariepinus*) exposed to mixture of atrazine and metolachlor in the laboratory. *Journal of Fisheries Sciences.com* 11.3: 48 – 54.

Adinortey, M. B., Sarfo, J. K. Quayson, E. T. Weremfo, A. Adinortey, C. A. Ekloh, W. and Ocran, J. 2012. Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. *Research Journal of MedicinalPlants*6.2: 171 – 179.

Aggarwal, A., Tandon, S., Singla, S. K. and Tandon, C. 2010. Diminution of Oxalate Induced Renal Tubular Epithelial Cell Injury and Inhibition of Calcium Oxalate Crystallization in vitro by Aqueous Extract of *Tribulus terrestris*: *International Brazilian journal of Urology*36.4: 480 – 489.

Ahilan, B., Nithiyapriyatharshini, A. and Ravaneshwaran, K. 2010.Influence of certain herbal additives on the growth, survival and disease resistance of goldfish, *Carassius auratus* (Linnaeus). *Tamilnadu Journal of Veterinary and Animal Science* 6.1: 5 – 11.

Ahmad M., Kaloo, Z. A., Ganai, B. A., Ganaie, H. A. and Singh, S. 2016. Phytochemical Screening of *Meconopsis aculeata* Royle an Important Medicinal Plant of Kashmir Himalaya: A Perspective. *Research Journal of Phytochemistry* 10: 1-9.

Ahmad, M. H. and Abdel-Tawwab, M. 2011. The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticuss* (L.) fingerlings. *Aquaculture* 314.1-4: 110-114.

Ahmed, Y. M., Idowu, T. A., Adedeji, H. A. and Sogbesan, O. A. 2015. Sex Reversal and Growth Performances in *Clarias gariepinus* (Burchell, 1822) Fry Treated with Dietary Genistein under Laboratory Conditions. *Journal of Agriculture and Food Technology* 5.5: 1-4.

Ahmed, G.U. Sultana, N. Shamsuddin M. and Hossain, M. B. 2013. Growth and production performance of monosex Tilapia (*Oreochromis niloticuss*) treated with with homemade feed in earthen mini ponds. *Pakistan Journal of Biological Sciences* 16.23: 1781 – 1785.

Akankali, J. A., Seiyaboh, E. I. and Abowei, J. F. N. 2011. Fish hatchery management in Nigeria. *Advance Journal of Food Science and Technology* 3.2: 144 – 154.

Akinrotimi, O. A. and Gabriel, U. U. 2012. Haematological profiles of *Clarias gariepinus* brood fish raised in water recirculating aquaculture system. *Advances Agricultural science and Engineering Research* 2: 97-103.

Akinrotimi, O. A., Agokei, E. O. and Aranyo, A. A. 2016. Changes in Blood Parameters of Tilapia Guineensis Exposed to Different Salinity Levels. *Journal of Environmental Engineering and Technology* 1.2: 4 – 12.

Akram1, M., Asif, H. M., Akhtar, N., Shah, P. A., Uzair, M., Shaheen, G., Shamim, T., Ali Shah, S. M., and Ahmad, K. 2011. *Tribulus terrestris* L.: A review article. *Journal of Medicinal Plants Research* 5.16: 3601-3605

Al-Asgah, N. A., Abdel-Warith, A. A., Younis, E. M. and Allam, H. Y. 2015. Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticuss* exposed to various concentrations of cadmium chloride. *Saudi Journal of Biological Science* 22.5: 543 – 550.

Al-Bayati, F. A. and Al-Mola, H. F. 2008. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *Journal of Zhejiang University Science B*: 9.2: 154 – 159.

Albuquerque, B. R., Prieto, M.A, Barreiro, M. F., Rodrigues, A., Curran, T. P., Barros, L. and Ferreira, I. C. F. R. 2017. Catechin-based extract optimization obtained from *Arbutus unedo* L. fruits using maceration/microwave/ultrasound extraction techniques. *Industrial Crops and Products* 95: 404 – 415.

Alhassan, E. H. E., Kombat, O. and Karim, D. 2018. Growth performance of the Nile Tilapia, *Oreochromis niloticuss* cultured in cages in two dams in the Bongo District of Ghana. *West African Journal of Applied Ecology* 26.1: 11 – 21.

Allen, K., Rachmi, A. F. and Cai, J. 2017. Nigeria: faster aquaculture growth needed to bridge fish demand–supply gap. *FAO Aquaculture Newsletter* 57: 36 – 37.

Alok, K. P., Rahman M. M. and Islam M. S. 2018. Effects of commercial feeds on the growth and carcass compositions of monosex Tilapia (*Oreochromis niloticuss*). *Journal of Fisheries and Aquatic Science* 13: 1-11.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. 2017. Phytochemicals: Extraction, isolation and identification of bioactive compounds from plant extracts. *Plants* 6.42: 23 p.

Amirshekari, T., Ziaei, N. Ghoreishi, S. M. and Esfandiarpour, E. 2015. The effects of adding aqueous extract and dried aerial part powder of *Tribulus terrestris* on productive performance and blood parameters of laying hens. *Journal of Applied Poultry Research* 25:145 – 155.

Ammar, N. M., El-Hawary, S. S. E., Mohamed, D. A., Afifi, M. S, Ghanem D. M. and Awad, G. 2018. Phytochemical and Biological Studies of *Tribulus terrestris* L. Growing in Egypt. *International Journal of Pharmacology* 14: 248 – 259.

Angelova S, Gospodinova Z, Krasteva M., Antov, G., Lozanov, V., Markov, T., Bozhanov, S., Georgieva, E. and Mitev V. 2013. Antitumor activity of Bulgarian herb *Tribulus terrestris* L. on human breast cancer cells. *Journal of BioScience and Biotechnology* 2: 25 – 32.

Antache, A., Cristea, V., Grecu, I., Dediu, L., Creţu, M. and Petrea, Ş. 2014. The influence of some phytobiotics on haematological and some biochemical indices at *Oreochromis niloticuss*Linnaeus, 1758. *Scientific Papers:Animal Science and Biotechnologies* 47.1: 192 – 199.

Anuradha, M., Pragyandip, P. D., Richa, K. and Murthy, P. N. 2010. Evaluation of neuropharmacological effects of ethanolic extract of *Clitorea Ternatea* flowers. *Pharmacology online* 1: 284-292.

Arendt, E. K., and Zannini, E. 2013. *Cereal Grains for the Food and Beverage Industries*. Woodhead Publishing, Cambridge. 485.

Arya, V., Thakur, N. M. and Kashyap, C. P. 2012. Preliminary Phytochemical Analysis of the Extracts of *Psidium* Leaves. *Journal of Pharmacognosy and Phytochemistry* 1.1: 1-5

Asad F., Ahmed, I., Saleem, M. and Iqbal, T. 2010. Hormonal masculinization and growth performance in Nile Tilapia (*Oreochromis niloticuss*) by androgen administration at different dietary protein levels. *International Journal of Agriculture and Biology* 12: 939–943.

Asche, F. 2008. Farming the Sea. *Marine Resource Economics* 23.4: 527 – 547.

Association of Official Analytical Chemists (AOAC) 2005. Official methods of analysis of AOAC International: W. Horwitz and G. W. Latimer Eds. 18th Ed. AOAC International. Arlington, VA, USA.

Assunção, T. O., Barros, C. M. M. R., Freitas, H. R. and Silva, T. H. 2017. *Tribulus terrestris* L. (zygophyllaceae): safety and effectiveness of steroidal metabolites. Retrieved on Feb, 2019 from https://www.researchgate.net/publication/320909030

Atanda, A. N. 2012. Fish species diversification in aquaculture for the success of the agriculture transformation agenda: the role of tilapia production. A paper presented at 2012 Annual public Lecture of the Fisheries Society of Nigeria (FISON), 21pp

Ayoola, S. O. 2010. Sustainable fish production in Africa. *African Journal of Food, Agriculture, Nutrition and Development* 10.5: 1-9

Azwanida, N. N. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants* 4.3:1-6.

Baba, E., Uluköy, G. and Mammadov, R. 2014. Effects of *Muscari comosum* extract on nonspecific immune parameters in Gilthead Seabream, *Sparus aurata* (L. 1758). *Journal of World Aquaculture Society* 45.2: 173-182

Babahajiani, P., Shokrollahi, B. and Gharibkhani, M. 2018. The effect of gokshura (*Tribulus terrestris)* and nettle root (*Urtica dioica*) extracts on growth rate and sex reversal in convict cichlid (*Cichlasoma nigrofasciatum*). *Iranian Journal of Fisheries Sciences* 17.3: 620 – 628.

Bai D., Wu X., Zhu G., Guo Y., Yang G., Ning B and Xing K., 2012. Astragalus polysaccharides enhance cellular immune response and disease resistance in yellow catfish. *The Israel Journal of Aquaculture-Bamidgeh*, IJA_ 64. 688, 7 p.

Bain, B. J., Lewis, S. M. and Bates, I. 2006. *Basic haematological techniques*. In S. M. Lewis, B. J. Bain, and I. Bates, Eds. Dacie and Lewis practical haematology, 10th edition. Churchill Livingstone Elsevier, Philadelphia. 26– 54pp

Ballard, T.S., Mallikarjunan, P., Zhou, K. and O'Keefe, S. 2010. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. *Food Chemistry* 120: 1185 – 1192.

Bamba, Y., Ouattara, A., Dacosta, K. S. and Gourène, G. 2008. Growth performances of juvenile *Oreochromis niloticuss* treated with with agricultural residues. *Sciences and Nature* 5.1: 89 – 99.

Bamba, Y., Ouattara, N., Soro, Y., Ouattara, A., Yao, K. and Gourène, G. 2014. Evaluation of production efficiency of Nile tilapia (*Oreochromis niloticuss* L.) treated with diets that contain crop residues in combination with cocoa bean shell and coconut oil cake in Côte d'Ivoire. *Livestock Research for Rural Development 6.189.* Retrieved Feb 18, 2019, from: http://www.lrrd.org/lrrd26/10/bamb26189.htm

Baroiller, J. F. and D'Cotta, H. 2016. The reversible sex of gonochoristic fish: insights and consequences. *Sexual Development* 10: 242–266.

Baroiller, J. F., D'Cotta, H., Bezault, E., Wessels, S. and Hoerstgen-Schwark, G. 2009. Tilapia sex determination: Where temperature and genetics meet. *Comparative Biochemistry Physiology* A 153: 8-30.

Baruah, L. D., Arya, A., Nalwade, V. and Devi, R. 2017. Development of polyherbal granules and its nutritional characterization. *Asian Journal of Dairyand Food Research* 36.3: 214- 221

Basaiyye, S. S., Naoghare, P. K., Kanojiya, S., Bafana, A., Arrigo, P., Krishnamurthi, K. and Sivanesan, S. 2018. Molecular mechanism of apoptosis induction in Jurkat E6-1 cells by *Tribulus terrestris* alkaloids extract. *Journal of Traditional and Complementary Medicine* 8: 410 – 419.

Begum, A., Mondal, S., Ferdous, Z., Zafar, M. A. and Ali, M. M. 2014. Impact of water quality parameters on monosex Tilapia (*Oreochromis niloticuss*) production under pond condition. *International Journal of Animal and Fisheries Science* 2.1: 14-21.

Belton, B. and Thilsted, S. H. 2014. Fisheries in transition: Food and nutrition security implications for the global South. *Global Food Security* 3: 59 – 66.

Benson, H. J., Gunstream, S. E., Talaro, A. and Talaro, K. P. 1989. *Anatomy and Physiology Laboratory Textbook*. Win. C. Brown/ McGraw-Hill Publisher Dubuque IOWA. 655 pp

Beveridge, M. C. M., Thilsted, S. H., Phillips, M. J., Metian, M., Troell, M. and Hall, S. J. 2013. Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology* 83.4: 1067 – 1084.

Bhatnagar, A. and Devi, P. 2013. Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Sciences* 3.6: 30 p.

Bhatnagar, A. and Singh, G. 2010. Culture fisheries in village ponds: a multilocation study in Haryana, India. *Agriculture and Biology Journal of North America* 1.5: 961-968

Bilale, A. M. and Teklie, A. T. 2017. Evaluation of growth performance of Nile tilapia (*Oreochromis niloticuss,* Linnaeus, 1752) with supplementary feeding of brewery waste in concrete ponds at Tehuledere district, Amhara Region, Ethiopia. *International Journal of Fisheries and Aquatic Studies* 5(4): 295-299

Bimakr, M. 2011. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food Bioproduct Processing* 89.1:67 – 72.

Bohlouli S. and Sadeghi, E. 2016. Growth performance and haematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings supplemented with dietary *Ferulago angulata* (Schlecht) Boiss. *Acta Veterinaria Brunensis* 85: 231-238

Bohm, B. A. and Kocipai-Abyazan, R. 1994. Flavonoid and condensed tannins from the leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*. *Pacific Science*48:458–463

Brody, J. R. and Kern, S. E. 2004 History and principles of conductive media for standard DNA electrophoresis. *Analytical Biochemistry* 333: 1–13.

Brummett, R. E., Lazard, J. and Moehl, J. 2008. African aquaculture; realizing the potential. *Food Policy* 33: 371 – 385.

Busari, A. O. 2018. Economic Analysis of Homestead Fish Farming in Olorunda Local Government Area, Osun State, Nigeria. *Nigerian Journal of Fisheries and Aquaculture* 6.2: 19 – 26

Caldini, N. N., Rebouças, T. V., Cavalcante, H. D., Martins, B. R. and Carmo, V. M. 2011. Water quality and Nile tilapia growth performance under different feeding schedules. *Acta Scientiarum Animal Sciences* 33.4: 427-430.

Cathrine, L. and Nagarajan, N. P. 2011. Preliminary phytochemical analysis and antibacterial activity of leaf extracts of *Vitex leucoxylon* L.F. *International Journal of Current Pharmaceutical Research*3.2: 71 – 73.

Çek, Ş., Turan, F. and Atik, E. 2007a. Masculinization of convict cichlid (*Cichlasoma nigrofasciatum*) by immersion in *Tribulus terrestris* extract. *Aquaculture International* 15.2: 109-119

Çek, S., Turan, F. and Atik, E. 2007b. The effects of Gokshura, *Tribulus terrestris* on sex reversal of guppy, *Poecilia reticulata*. *Pakistan Journal of Biological Sciences* 10.5: 718-725.

Chakraborty S. B. 2017. Evaluation of plants extracts on sex reversal and growth of Nile tilapia Final Progress Report (2013 - 2016). Retrieved on 6th Nov, 2018 from https://www.semanticscholar.org/paper/Evaluation-of-plants-extracts-on-sex-reversal-and-(-Chakraborty/2da8c3f2ec660a487cf50b622eb7942859c4654e

Chakraborty, B. S., Molnar, T. and C. Hancz, 2012. Effects of methyltestosterone, tamoxifen, ganistein, and *Basella alba* extract on masculinization of guppy (*Poecilia reticulata*). *Journal of Applied Pharmaceutical Science* 2.12: 48-52.

Chakraborty, S. B. and Banerjee, S., 2009. Culture of monosex Nile Tilapia under different traditional and non-traditional methods in India; *World Journal of Fish and Marine Sciences* 1.3: 212-217

Chakraborty, S. B. and Hancz, C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistresss agents in finfish culture. *Reviews in Aquaculture* 3: 103-119.

Chakraborty, S. B., Horn, P. and Hancz, C. 2014. Application of phytochemicals as growth promoters and endocrine modulators in fish culture. *Reviews in Aquaculture* 6: 1-19.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S. and Abert-Vian, M. 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasononics Sonochemistry* 34:540–560

Chhatre, S., Nesari, T., Somani, G. Kanchan, D. and Sathaye, S. 2014. Phytopharmacological overview of *Tribulus terrestris*. *Pharmacognosy Review* 8.15: 45-51

Chin, F.S., Chong, K.P. Markus, A. and Wong, N.K. 2013. Tea polyphenols and alkaloids content using soxhlet and direct extraction methods. *World Journal of Agricultural Sciences* 9.3: 266 – 270.

Choudhary, H. R. and Sharma, B. K. 2018. Impact of Nile tilapia (*Oreochromis niloticuss*) feeding on selected water quality parameters. *Journal of Entomology and Zoology Studies* 6.5: 2371-2377

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International* 18.3: 403-414.

Cline, D. 2012. Water Quality in Aquaculture. Retrieved Jan, 29, 2019 from: https://articles.extension.org/pages/58707/water-quality-in-aquaculture

Cnaani, A., and Levavi-Sivan, B. 2009. Sexual development in fish, practical applications for aquaculture. *Sexual Development* 3.2-3: 164-175.

Cockell, K. A., Hilton, J. W. and Bettger, W. J. 1992. Hepatobiliary and hematological effects of dietary disodium arsenate heptahydrate in juvenile rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology*, *Part C: Comparative Pharmacology* 103:453 – 458.

Colville, J. 2002. Blood chemistry. In: Hendrix CM, editor. Laboratory procedures for veterinary technicians. 4th ed. St. Louis (MO): Mosby; p. 75–103.

Conde-Hernández, L. A., Espinosa-Victoria, J.R., Trejo, A. and Guerrero-Beltrán, J. Á. 2017. CO_2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*) *Journal of Food Engineering*200:81–86.

Ćujić, N., Šavikin, K., Janković, T., Pljevljakušić, D., Zdunić, G. and Ibrić, S. 2016. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry*194: 135 – 142.

Dada, A. A. 2015. Use of fluted pumpkin (*Telfairia occidentalis*) leaf powder as feed additive in African catfish (*Clarias gariepinus*) fingerlings. *International Journal of Biological and Chemical Sciences* 9.1: 301-307.

Dal'Bó, G. A., Sampaio, F. G. Losekann, M. E. Queiroz, J. F. Luiz, A. J. B. Wolf, V. H. G. Gonçalves, V. T. Carra, M. L. 2015. Hematological and morphometric blood value of four cultured species of economically important tropical foodfish. *Neotropical Ichthyology* 13.2: 439 – 446.

Das, K., Tiwari, R. K. S. and Shrivastava, D. K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research* 4.2: 104 – 111.

Das, R., Rather, M. A., Basavaraja, N., Sharma, R. and Udit, U. K. 2012. Effect of non-steroidal aromatase inhibitor on sex reversal of *Oreochromis mossambicus* (Peter, 1852). *The Israel Journal of Aquaculture-Bemidgeh* 64: 64-69

Dastagir, G., Hussain, F and Khattak, K. F. 2014. Nutritional Evaluation of Plants of Family Zygophyllaceae And Euphorbiaceae. *Pakistani Journal of Botany* 46.5: 1703-1707

Dastagir, G., Hussain, F., Khattak, K. F. and Khanzadi, H. 2013. Proximate analysis of plants of family Zygophyllaceae and Euphorbiaceae during winter. *Sarhad Journal of Agriculture* 29.3: 395 – 400

Dauda, A. B., Yakubu, S. O. Oke, A. O. 2014. Curbing the menace of prolific breeding in "aquatic chicken" (Tilapia): A way out to improve fish production in Nigeria. *New York Science Journal* 7.4: 112-118

De Silva, G. O., Abeysundara, A. T. and Aponso, M. M. W. 2017. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products* 5.2: 29 – 32.

Dein, F.J., 1984. Laboratory Manual of Avian Haematology. Association of Avian Veterinarian, East North Port, USA. 38pp.

Dereje, D., Prabha, L. D., Sreenivasa, V. and Abebe, G. 2015. The growth performance of Nile Tilapia in earthen ponds located at different altitudes of Toke Kutaye Woreda, Ethiopia. *International Journal of Aquaculture* 5.34: 1-7.

Dergal, N. B., Scippo, M., Degand, G., Gennotte, V., Mélard, C. and Abi-Ayad, S. E. 2016. Monitoring of 17α-methyltestosterone residues in tilapia's (*Oreochromis niloticuss*) flesh and experimental water after its sex reversal. *International Journal of Biosciences* 9.6: 101 – 113.

Dhanalakshmi, J., Senthamarai, S. V. and Selvi, S. 2016. Anticancer and cytotoxic potential of ethanolic extract of *Tribulus terrestris* on hela cell lines. *International Research Journal of Biological Sciences* 5.6: 37 - 42.

Dhanani, T., Shah, S., Gajbhiye, N. A. and Kumar, S. 2013. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry* 10.1: S1193 – S1199.

Dhas, S. A., Selvaraj, T., Citarasu, T., Punitha, S. M. J. and Babu, M. M. 2015. Effect of supplemented diet with maturation plant extract on reproductive performance of Etroplus suratansis. *Aquaculture Reports, 2*: 58-62.

Divya, M. K., Dharmapal, S., Achuthan, C. R. and Babu, T. D. 2014. Cytotoxic and antitumor effects of *Tribulus terrestris* L fruit methanolic extract. *Journal of Pharmacognosy and Phytochemistry* 3.2: 1-4.

Diyaware, M. Y., Haruna, A. B. and Abubakar, K. A. 2013. Some haematological parameters of inter-generic hybrid of African catfish (*Clarias anguillaris x Heterobranchus bidorsalis*) juveniles and their pure lines in North Eastern Nigeria. *Journal of Fisheries and Aquatic Science* 8.1: 33 – 42.

Do, J., Choi, S., Choi, J. and Hyun, J. S. 2013. Effects and mechanism of action of a *Tribulus terrestris* extract on penile erection. *Korean Journal of Urology* 54: 183-188

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H. Soetaredjo, F. E., Ismadji, S. and Ju, Y. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic. Journal of Food and Drug Analysis* 22.3: 296 – 302.

Du, G., Sun, L., Zhao, R., Du, L., Song, J., Zhang, L., He, G., Zhang, Y., Zhang, J. 2016. Polyphenols: Potential source of drugs for the treatment of ischaemic heart disease. *Pharmacology and Therapeutics* 162: 23 – 34.

Duru, M. 2005. Investigation of possible anabolic effects of Yohimbe Bark (*pausinystalia yohimbe*) and iron thistle (*Tribulus terrestris*) extracts on growth performance and body composition of broiler chickens. Master's thesis, Mustafa Kemal University, Institute of Science and Technology (Unpublished), Hatay. 237 pp.

Dwivedi, D. and Sengar, N. 2018. Investigation of phytochemical constituents from *Tribulus terrestris* roots, leaves and fruits. *Journal of Chemistry and Chemical Sciences* 8.1: 55 - 58.

Ebrahim, N., Kershi, R. M. N, Awadh, A. A.,Ahmeda. T. A., and Ramzy, M. 2013. Antioxidant Activity and Anthocyanin Content in Flower of *Mirabilis jalab* L. Collected from Yemen. *World Applied Sciences Journal* 29: 247-251.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. 2005. Phytochemical constituents of some Nigerian medicinal plant. *African Journal of Biotechnology* 4: 685 – 688.

Edori, O. S., Dibofori-Orji, A. N. and Edori, E. S. 2013. Biochemical changes in plasma and liver of *Clarias gariepinus* exposed to paraquat. *IOSR Journal of Pharmacy and Biological Sciences* 8.2: 35 – 39.

Ejikeme, C. M., Ezeonu, C. S. and Eboatu, A. N. 2014. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *European Scientific Journal* 10.18: 247–270.

Ejiola, M. T. and Yinka, O. F. 2012. Comparative cost structure and yield performance analysis of upland and mangrove fish farms in Southwest, Nigeria. *International Journal of Agricultural Management and Development* 2.3:187 – 198.

Ekor, M. 2013. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology* 4.177: 10 p.

Ekubo, A. A. and Abowei, J. F. N. 2011. Review of some water quality management principles in culture fisheries. *Research Journal of Applied Sciences, Engineering and Technology* 3.2: 1342 – 1357.

Ekunwe, P. A., Emokaro, C. O. 2009. Technical efficiency of catfish farmers in Kaduna, Nigeria *Journal of Applied Sciences Research* 5.7: 802 – 805.

Elekwachi, L. O. 2018. Investigating the level of awareness of fish farming in Delta state, Nigeria. *International Journal of Scientific and Engineering Research* 9.8: 1432 – 1445.

El-Greisy, Z. A. and El-Gamal, A. E. 2012. Monosex production of tilapia, *Oreochromis niloticuss* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. *The Egyptian Journal of Aquatic Research*38.1: 59 – 66.

El-Sayed, M. A. B., Abdel-Aziz, H. E. S. and Abdel-Ghani, M. H. 2012. Effects of phytoestrogens on sex reversal of Nile tilapia (*Oreochromis niloticuss*) larvae treated with diets treated with 17α-methyltestosterone. *Aquaculture* 360: 58-63.

El-Shaibany, A., AL-Habori, M., Al-Tahami, B. and Al-Massarani, S. 2015. Anti-hyperglycaemic Activity of *Tribulus terrestris* L Aerial Part Extract in Glucose-loaded Normal Rabbits. *Tropical Journal of Pharmaceutical Research* 14.12: 2263 – 2268.

El-Sherif, M. S. and El-Feky, A. M. 2009. Performance of Nile Tilapia (*Oreochromis niloticuss*) fingerlings: influence of different water temperatures. *International Journal of Agriculture Biology* 11: 301-305

El-Zaeem, S. Y. and Salaam, G. M. 2013. Production of genetically male tilapia through interspecific hybridization between *Oreochromis niloticuss* and *O. aureus*. *Iranian Journal of Fisheries Science.* 12.4: 10 p

Ernst, W. H. and Tolsma, D. J. 1988. Dormancy and germination of semi-arid annual plan species, *Tragus berteronianus* and *Tribulus terrestris*. *Flora* 181.3/4: 243 – 251.

Fagbenro, O. A., Jegede, J. and Fasasi, O. S. 2010. Tilapia aquaculture in Nigeria. *Applied Journal of Tropical Agriculture* 15: 49-55.

Fagbenro, O.A. 2004. Predator control of overpopulation in cultured tilapias and the alternative uses for stunted tilapias in Nigeria. Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, pp.634-647. (R.B. Bolivar, G.C. Mair and K. Fitzsimmons, eds.). Manila, Philippines. September 12-16, 2004.

Fagbenro, O. A., Adeparusi, E. O. and Adeyemi, J. W. 2013. Haematological profile of blood of African Catfish (*Clarias gariepinus*, Burchell, 1822) fed sunflower and sesame meal based diets. *Journal of Fisheries and Aquatic Science* 8.1:80 – 86.

Fagbuaro, O., Iwalaye, O. A. and Ariyo, A. F. 2016. Heamatological and serum biochemical profile of Nile tilapia, O*reochromis niloticuss* from Ero dam in Ikun Ekiti, Ekiti State Nigeria. *American Journal of Research Communication* 4.4: 192 – 205.

Falcão, M. A., Scopel, R., Almeida, R. N., do Espirito-Santo, A. T., Franceschini, G., Garcez, J. J., Vargas, R. M. F. and Cassel, E. 2017. Supercritical fluid extraction of vinblastine from *Catharanthus roseus*. *Journal of Supercritical Fluids* 129: 9–15.

Falconer, D. S., and Mackay, T. F. C. 1996. Introduction to Quantitative Genetics, Ed 4. Longmans Green, Harlow, Essex, UK http://www.genetics.org/content/167/4/1529

Farahi, A., Kasiri, M., Sudagar, M., Soleimani, I. M. and Zorriehzahra, S. M. J. 2012. Effect of dietary supplementation of *Melissa officinalis* and *Aloe vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). *Online Journal of Animal and Feed Research* 2:1 – 5.

Farooq, S. A., Farook, T. T. and Al-Rawahy, S. H. 2012. bioactive compounds from T*ribulus terrestris L*. (Zygophyllaceae). *Natural Products and Their Active Compounds*. M. Essa, A. Manickavasagan, and E. Sukumar Eds. Nova Science Publishers, Inc. 2012. Chapter 13: 245 – 268.

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N. and Kadri, A. 2017. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Food Science and Technology Campinas*37.3: 483 – 492.

Felicitta, J., Manju, R. A., Ronald, J., Sakthika, T., Nagarajan, R. and Chelladurai, G. 2013. The effect of different concentrations garlic (*Allium sativum*) and onion

(*Allium cepa*) on growth, survival, and hematology of juvenile tilapia (*Oreochromis mossambicus*). *TheIsreali Journal of Aquaculture- Bamidgeh*, IJA_ 65.822: 5p.

Ferdous, Z. and Ali, M. M. 2011. Optimization of hormonal dose during masculinization of tilapia (*Oreochromis niloticuss*) fry. *Journal of the Bangladesh Agricultural University* 9.2: 359 – 364.

Ferreira, P. M. F., Caldas, D. W., Salaro, A. L. Sartori, S. S. R., Oliveira, J. M., Cardoso, A. J. S. and Zuanon, J. A. S. 2016. Intestinal and liver morphometry of the yellow tail tetra (*Astyanax altiparanae*) treated with with oregano oil. *Anais da Academia Brasileira de Ciências* 88.2: 911-922.

Fishery Committee for the West Central Gulf of Guinea (FCWC). 2018. Nigeria: Aquaculture experts want increased Tilapia production to boost export. Retrieved on 8th Aug, 2019 from https://www.fcwc-fish.org/publications/news-from-the-region/1501-nigeria-aquaculture-experts-want-increased-tilapia-production-to-boost-export.html

Fishery Committee for the West Central Gulf of Guinea (FCWC). 2016. Nigeria fishery statistics – 2016, Summary report. Retrieved on Nov. 19, 2017 from: https://www.fcwc-fish.org/fisheries/statistics/nigeria.html

Food and Agriculture Organization of the United Nations (FAO). 2003. Food energy-methods of analysis and conversion factors. Food and Nutrition Paper 77, Food and Agriculture Organization of the United Nations, Rome, Italy, pp 1-93

Food and Agriculture Organization of the United Nations, (FAO). 2008. Food and Agricultural Organization: Fisheries Report No 12. p. 5.

Food and Agriculture Organization of the United Nations (FAO). 2010. The State of World Fisheries and Aquaculture, 2010. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), 197 pp. retrieved on March 7, 2014 fromhttp://www.fao.org/docrep/013/i1820e/i1820e.pdf

Food and Agriculture Organization of the United Nations (FAO). 2012. The State of World Fisheries and Aquaculture 2012. FAO, Rome, p209. FAO/GLOBEFISH, 2013. GLOBEFISH highlights, a quarterly update on world seafood market 3/2013. FAO, Rome, p. 61.

Food and Agriculture Organization of the United Nations (FAO). 2013. *Global Aquaculture Production 2013*; FAO: Rome, Italy, 2013. 102pp.

Food and Agriculture Organization of the United Nations (FAO). 2014. The State of the World Fisheries and Aquaculture: Opportunities and challenges. Retrieved Dec, 26, 2017 fromhttps://www.eumofa.eu/documents/20178/66921/FAO_SOFIA_EN_2014.pdf/c30ef7cc-476a-493a-97f9-4a514555cbb3

Food and Agriculture Organization of the United Nations (FAO). 2016a. The State of the World Fisheries and Aquaculture: contributing to food security and food for all. Retrieved Dec, 26, 2017 from http://www.fao.org/fishery/sofia/en

Food and Agriculture Organization of the United Nations (FAO). 2016b. Cultured aquatic species information programme: *Clarias gariepinus*. Fisheries and Aquaculture Department, Food and Agricultural Organization of the United Nations, Rome. 204pp.

Food and Agriculture Organization of the United Nations, (FAO). 2017. FAO Aquaculture Newsletter No. 57. http://www.fao.org/3/a-i7851e.pdf

Food and Agriculture Organization of the United Nations (FAO). 2018. The State of World Fisheries and Aquaculture, Meeting the Sustainable Development Goals. 227pp.

Francesco, F., Satheeshkumar, P. Kumar, D. S. Faggio, C. and Giuseppe, P. 2012. A comparative study of hematological and blood chemistry of Indian and Italian grey Mullet (*Mugil cephalus Linneaus 1758*), *Herbert Open Access Journal Biology* 5 p.

Francis, G., Kerem, Z., Makkar, H. and Becker, K. 2003. The biological action of saponins in animal systems: A review. *The British journal of nutrition* 88: 587 – 605.

Froese, R. and Pauly, D. 2004. FishBase DVD. Penang, Malaysia: Worldfish Center. Online at www.fishbase.org

Froese, R. and Pauly, D. 2011. Invasive species compendium. FishBase. http://www.fishbase.org

Fuentes-Silva, C., Soto-Zarazua, G. M., Torres, P. I. and Flores-Rangel, A. 2013 Male tilapia production techniques: A mini-review. *African Journal of Biotechnology* 12.36: 5496-5502.

Gabriel, N. N. 2019. Review on the progress in the role of herbal extracts in tilapia culture. *Journal of Cogent Food and Agriculture* 5: 1619651. Retrieved June 26, 2019 from https://doi.org/10.1080/23311932.2019.1619651

Gabriel, U. U., Akinrotimi, O. A. and Eseimokumo, F. 2011. Haematological responses of wild Nile Tilapia *Oreochromis niloticuss* after acclimation to captivity. *Jordan Journal of Biological Sciences* 4.4: 225 – 230

Gabriel, N. N., Qiang, J., He, J., Ma, Y. X., Kpundeh, M. D. and Xu, P. 2015a. Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). *Fish and Shellfish Immunol*ogy 44.2: 504 – 514.

Gabriel, N. N., Qiang, J., Kpundeh, M. D. and Xu, P. 2015b. Use of herbal extracts for controlling reproduction in Tilapia culture: trends and prospects - a Review. *The Israeli Journal of Aquaculture - Bamidgeh*_67.2015.1178: 22pp.

Gabriel, N. N., Qiang, J., Ma, X. Y., He, J., Xu, P. and Omoregie, E. 2017. Sex reversal effect of dietary *Aloe vera* (Liliaceae) on genetically improved farmed Nile Tilapia fry. *North American Journal of Aquaculture* 79.1: 100-105

Gabriel, N. N., Wilhelm, M. R., Habte-Tsion, H. M., Chimwamurombe, P., Omoregie, E., Iipinge, L. N. and Shimooshili, K. 2019. Effect of dietary *Aloe vera* polysaccharides supplementation on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. *International Aquatic Research* 11.1: 57 – 72.

Ganzera, M., Bedir, E. and Khan, I. A. 2001. Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. *Journal of Pharmaceutical Sciences* 90.11: 1752–1758.

Gauthaman, K. and Adaikan, P. G. 2008. The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction – an evaluation using primates, rabbit and rat. *Phytomedicine* 15.1: 44–54

Gauthaman, K., and Ganesan, A. P. 2008. The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction–an evaluation using primates, rabbit and rat. *Phytomedicine* 15.1-2: 44-54.

Ghanbari, A., Moradi, M. Raoofi, A. Falahi, M. and Seydi, S. 2016. *Tribulus terrestris* hydroalcoholic extract administration effects on reproductive parameters and serum level of glucose in diabetic male rats. *International Journal of Morphology* 34.2: 796 – 803.

Ghosal, I. and Chakraborty, S. B. 2014a. Effects of the aqueous leaf extract of *Basella alba* on sex reversal of Nile Tilapia, *Oreochromis niloticuss. IOSR Journal of Pharmacy and Biological Sciences* 9.2: 162 – 164

Ghosal, I. and Chakraborty, S. B. 2014b. Effects of the aqueous seed extract of *Tribulus terrestris* on sex reversal of Nile Tilapia, *Oreochromis niloticuss*. *Indian Journal of Applied Research* 4.9: 549 – 561

Ghosal, I., Mukherjee, D., Hancz, C and Chakraborty, S. B. 2015. Efficacy of *Basella alba* and *Tribulus terrestris* extracts for production of monosex Nile tilapia, *Oreochromis niloticuss*. *Journal of Applied Pharmaceutical Science* 5.8: 152-158

Ghosal, I., Mukherjee, D., Hancz, C and Chakraborty, S. B. 2016. Production of monosex Nile tilapia, *Oreochromis niloticuss* by dietary and immersion treatment with *Basella alba* leaves and *Tribulus terrestris* seeds. *International Journal of Fisheries and Aquatic Studies* 4.1: 358 – 363.

Ghosian, M. M. H., Khalili, M., Maleki, M. and Ahmad, A. M. E. 2013. The Effect of oral feeding of *Tribulus terrestris* L. on sex hormone and gonadotropin levels in addicted male rats. *International Journal of Fertility and Sterility* 7.1: 57 – 62.

Gincy M. S., Mohan, K. and Indu, S. 2014. Comparitive phytochemical analysis of medicinal plants namely *Tribulus terrestris, Ocimum sanctum, Ocimum gratissinum, Plumbago zeylanica. European Journal of Biotechnology and Bioscience* 2.5: 38-40

Golestan, G., Salati, A. P., Keyvanshokooh, S., Zakeri, M. and Moradian, H. 2015. Effect of dietary *Aloe vera* on growth and lipid peroxidation indices in rainbow trout (*Oncorhynchus mykiss*). *Veterinary Research Forum* 6.1: 63–67.

Grigorova, S., Kashamov, B. Sredkova, V. Surdjiiska S. and Zlatev, H. 2008. Effect of *Tribulus terrestris* extract on semen quality and serum total cholesterol content in white plymuth rock-mini cocks. *Biotechnology in Animal Husbandry* 24.3-4: 139 – 146.

Grigorova, S., Abadjieva, D. Nikolova,M. and Penkov, D. 2009. The effect of *Tribulus terrestris* extract on egg yolk lipids and serum cholesterol content in guinea fowls. *Biotechnology in Animal Husbandry* 25: 1109 – 1115.

Grigorova, S., Nikolava, M. Penkov, D. and Gerzilov, V. 2014. Egg yolk lipids change in Japanese quail given *Tribulus terrestris* extract. *Bulgarian Journal of Agricultural Science* 20.6: 1472 – 1476.

Guerrero III, R.D. 1975. Use of androgens for the production of all male Tilapia aurea (Steindachner). *Transactions of the American Fisheries Society* 104:342–348.

Gültepe, N., Acar, Ü., Kesbiç, O. S., Yılmaz, S., Yıldırım, Ö. and Türker, A. 2014. Effects of dietary *Tribulus terrestris* extract supplementation on growth, feed utilization, hematological, immunological and biochemical variables of Nile Tilapia *Oreochromis niloticuss. The Israeli Journal of Aquaculture* 66.1024: 12pp

Guo, Z. Luo, X. Liang, J. Yang, Z, Ai, X. 2015. Comparative study on extraction of febrifugine from traditional Chinese medicine *Dichroa febrifuga* by reflux method and ultrasonic method. *Shizhen Guoyi Guoyao* 26.6:1532–1533.

Hall, S. J., Delaporte, A. Phillips, M. J. Beveridge, M. and O'Keefe, M. 2011. Blue frontiers: managing the environmental costs of aquaculture. The WorldFish Center, Penang, Malaysia. Retrieved on Jan 26, 2017 from https://www.conservation.org/publications/documents/BlueFrontiers_aquaculture_report.pdf

Hamid, A. S. H., Ahmed, M. F. A., Mohammed, A. M. I. and Ali, M. S. I. 2013. Physical and chemical characteristics of blood of two fish species (*Oreochromis niloticuss* and *Clarias lazera*). *World's Veterinary Journal* 3.1: 17 – 20.

Hammoda, H. M., Ghazy, N. M., Harraz, F. M., Radwan, M. M., El-Sohly, M. A. and Abdallah, I. I. 2013. Chemical constituents from *Tribulus terrestris* and screening of their antioxidant activity. *Phytochemistry* 92: 153-159.

Handa, S. S., Khanuja, S. P. S., Longo, G. and Rakesh, D. D. 2008. Extraction technologies for medicinal and aromatic plants, (1stedn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. ICS-UNIDO, AREA Science Park Padriciano 99, 34012 Trieste, Italy. 266pp.

Harbone, J. B. 1973. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.*Chapman and Hall Ltd 11 New Fetter Lane, London EC4P 4EE Fakenham Press LimitedFakenham, Norfolk 286pp.

Harikrishnan, R., Belasundaram, C. and Heo, M. S. 2010. Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. *Fish and Shellfish Immunology* 28.2: 354 – 361.

Hassan, M. S. and Soltan, M. A. 2016. Evaluation of essential oil of fennel and garlic separately or combined with *Bacillus licheniformis* on the growth, feeding behaviour, hemato-biochemical indices of *Oreochromis niloticuss* (L.) Fry. *Journal of Aquaculture Research and Development* 7:422: 8pp

Hassan, L. G., Umar, K. J. and Usman, A. 2005. Nutrient content of the leaves of *Tribulus terrestris* ("Tsaida"). *Journal of Tropical Biosciences* 5.6: 77 – 82.

Hemalatha, S. and Hari, R. 2015. Fertility enhancing effect of saponin rich butanol extracts of *Tribulus terrestris* fruits in male albino rats. *International Journal of Pharmaceutical and Clinical Research* 7.1: 36 – 43.

Hepsibah, A. H. and Jothi, G. J. 2017. A comparative study on the effect of solvents on the phytochemical profile and biological potential of *Ormocarpum cochinchinense* Auct. Non (Lour.) Merrill. *International Journal of Pharmacy and Pharmaceutical Sciences* 9.1: 67 – 72.

Hossain, M. A., Al-Toubi, W. A. S., Weli, A. M., Al-Riyami, Q. A. and Al-Sabahi, J. N. 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science* 7.4: 181-188.

Hrubec, T. C. and Smith, S. A. 2010. Hematology of fishes. In: D. J. Weiss and K. J. Wardrop Eds. Schalm's veterinary hematology. 6th ed. Ames, Iowa, Wiley-Blackwell. 994 – 1003.

Hu, H., Mai, K., Zhang, Y., Ai, Q., Xu, W. and Zhang, W. 2014. Effects of dietary xylan on growth performance, digestive enzyme activity and intestinal morphology of juvenile turbot (*Scophthalmus maximus* L). *Israeli Journal of Aquaculture-Bamidgeh* 67.1115: 10p.

Huang, Y., Xiao, D., Burton-Freeman, B. M. and Edirisinghe, I. 2016. *Reference Module in Food Science* Chemical Changes of Bioactive Phytochemicals during Thermal Processing. https://doi.org/10.1016/B978-0-08-100596-5.03055-9

Ilodibia, C. V., Ugwu, R. U., Okeke, C.U., Ezeabara, C. A., Okeke, N. F., Akachukwu, E. E. and Aziagba, B. O. 2014. Determination of proximate composition of various parts of two *Dracaena* species. *International Journal of Botany* 10: 37-41.

Immanuel, G., Uma, R. P., Iyapparaj, P., Citarasu, T., Peter, S.M., Babu, M.M. and Palavesam, A. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. *Journal of Fish Biology* 74.7: 1462 – 1475.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S. Moharil, M. P. and Khelurkar, V. C. 2017. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry* 6.1: 32 – 36.

Ismaiel, S. I., Farouk, S. M., El-Ramady R. A. and Khalil, W. F. 2017. Ameliorative impacts of *Tribulus terrestris* against ivermectin-induced hepato-renal toxicity in rabbit: Pharmacological and histopathological study. *American Journal of Animal and Veterinary Sciences* 12.1: 8 – 16.

Ivanova, A., Serly, J. Dinchev, D. Ocsovszki, I. Kostova, I. and Molnar, J. 2009. Screening of some saponins and phenolic components of *Tribulus terrestris* and *Smilax excelsa* as MDR modulators. *In vivo* 23: 545 – 550.

Janalizadeh, E., Manoucheri, H. and Changizi, R. 2018. A comparison with *Tribulus terrestris* extract fish immersion and bioincapsulation of enriched artemia on sex reversing of fighter fish (*Betta splendens*). *International Journal of Ornamental Aquatics Research* 1.1: 9 – 17.

Janalizadeh, E., Manoucheri, H. and Changizi, R. 2019. A comparison with *Tribulus terrestris* extract fish immersion and bioincapsulation of enriched artemia on sex

reversing of fighter fish (*Betta splendens).* *Journal of Survey in Fisheries Sciences 5.2: 89 – 97.*

Jegede, T. 2009. Population control of farm-raised tilapias (*Oreochromis niloticuss* and *Tilapia zillii*) using pawpaw seed and neem leaf meals as reproduction inhibitors. Ph.D. Thesis, Treated witheral University of Technology, Akure, Nigeria. 151pp.

Jegede, T. 2010. Control of reproduction in *Oreochromis niloticuss*(Linnaeus 1758) using *Hibiscus rosasinensis* (Linn.) leaf meal as reproduction inhibitor. *Journal of Agricultural Science* 2.4: 149-154.

Ji, J., Lu, C., Kang, Y., Wang, G. X. and Chen, P. 2012. Screening of 42 medicinal plants for in vivo anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Parasitology Research*111: 97 – 104.

Jim, F. Garamumhango, P. and Musara, C. 2017. Comparative Analysis of Nutritional Value of *Oreochromis niloticuss* (Linnaeus), Nile Tilapia, Meat from Three Different Ecosystems. *Journal of Food Quality*. Retrieved on Feb. 15, 2019 from https://10.1155/2017/6714347 8pp.

Jimoh, W.A., Shittu, M.O. Ayeloja, A. A. Ajasin, F. O. Okemakin, F. Y. Abdusalami, S. A. and Adekunle, O. F. 2015. Some haematological and biochemical profile of blood of Nile Tilapia (*Oreochromis niloticuss*) treated with on diets that contain watermelon (*Citrullus lanatus*) seedmeal. *Bayero Journal of Pure and Applied Sciences* 8.1: 109 – 114.

Jin, S., Yang, M., Kong, Y., Yao, X., Wei, Z., Zu, Y. and Fu, Y. 2011. Microwave-assisted extraction of flavonoids from *Cajanus cajan* leaves. *Zhong cao yao* 42.11: 2235 – 2239.

Jindal, A., Kumar, P. and Gautam, K. 2013. Evaluation of antibiotic potential of *Tribulus terrestris* L. against some pathogenic microorganisms. *International Journal of Green Pharmacy* 7: 102 – 105.

Job, B. E., Antai, E. E., Inyang-Etoh, A. P., Otogo, G. A. and Ezekiel, H. S. 2015. Proximate Composition and Mineral Contents of Cultured and Wild Tilapia (*Oreochromis niloticuss*) (Pisces: Cichlidae) (Linnaeus, 1758). *Pakistan Journal of Nutrition* 14.4: 195 – 200.

Joseph, B. and Raj, S. J. 2011. Effect of curacron toxicity on aminotransferases (ALT and AST) in the serum of the fish *Cyprinus Carpio*. *International Journal of Biological Chemistry* 5.3: 207 – 211.

Jovanović, A. A., Đorđević, V. B., Zdunić, G. M., Pljevljakušić, D. S., Šavikin, K. P., Gođevac, D. M. and Bugarski, B. M. 2017. Optimization of the extraction process of polyphenols from *Thymus serpyllum* L. herb using maceration, heat- and ultrasound-assisted techniques. *Separation and Purification Technology* 179:369–380.

Kabir, S., Khanzada, A. K., Baloch, M., Khaskheli, A. R. and Shaikh, W. 2015. Determination of total protein contents from medicinal plants (Zygophyllaceae) family found in Pakistan. *Sindh University Research Journal (Science Series).* 47.1: 41 – 44.

Kamali-Sanzighi, M., Akrami, R., Ghelichi, A. and Shamloofar, M. 2018. Partial replacement of plant sources by waste date (P*honeix dactylifera*) in the diet of fingerling common carp (*Cyprinus carpio*) on growth performance, feed utilization, hematological parameters and resistance to stress. *Turkish Journal of Fisheries and Aquatic Sciences* 19.9: 775 – 784.

Kamaruzzaman, N., Nguyen, N. H., Hamzah, A. and Ponzoni, R. W. 2009. Growth performance of mixed sex, hormonally sex reversed and progeny of YY male tilapia of the GIFT strain, *Oreochromis niloticuss*. *Aquaculture Research* 40.6: 720 – 728.

Kareem, Z. H., Yasser, M. A., Christianus, A., Karim, M. and Romano N. 2016. Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile

tilapia (*Oreochromis niloticuss*) and their resistance to *Streptococcus agalactiae* infection. *FishPhysiology and Biochemistry*42:757–769.

Karpagam, B. and Krishnaveni, N. 2014. Effect of supplementation of selected plant leaves as growth promoters of Tilapia fish (*Oreochromis mossambicus*). *Research Journal of Recent Sciences* 3: 120 – 123.

Kavitha, P. and Subramanian, P. 2011. Effect of *Tribulus terrestris*on monosex production in *Poecilia latipinna*. *Current Science* 101.1: 100 – 104.

Kavitha, P., Ramesh, R., Bupesh, G., Stalin, A., and Subramanian, P. 2011. Hepatoprotective activity of *Tribulus terrestris* extract against acetaminophen-induced toxicity in a freshwater fish (*Oreochromis mossambicus*). *In Vitro Cellular and Developmental Biology-Animal* 47.10: 698-706.

Kavitha, P., Ramesh, R., and Subramanian, P. 2012. Histopathological changes in *Poecilia latipinna* male gonad due to *Tribulus terrestris* administration*In Vitro Cellular and Developmental Biology. Animal* 48.5: 306 – 312.

Kawarazuka, N. and Béné, C. 2011. The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence, 14: 1927-1938. DOI: 10.1017/S1368980011000814.

Kefi, A. S., Kang'ombe, J. Kassam, D. Katongo, C. 2013. Effect of 17 a-methyl testosterone on haematology and histology of liver and heart of *Oreochromis andersonii* (Castelnau, 1861)*Journal of Marine Science: Research and Development* 3: 130 doi:10.4172/2155-9910.1000130

Khakong, S., Leelarasamee, K., Suwannasang, A., and Sukkasem, N. 2011. Effects of natural extracts from mangosteen leaves on sex-reversal in Nile tilapia (Oreochromis *niloticus*s). *KhonKaen Agriculture Journal* 39: 53-58.

Khan S. A., Aslam, R. and Makroo, H. A. 2018. High pressure extraction and its application in the extraction of bio-active compounds: A review. *Journal of Food Engineering* 42.1: 1-15.

Kiran, B., Lalitha, V. and Raveesha, K. A. 2011. in vitro Evaluation of Aqueous and Solvent extract of *Tribulus terrestris* L. leaf against Human bacteria. *International Journal of PharmTech Research* 3.3: 1897 – 1903.

Kiriyakit, A. 2014. Efficacy of red Kwao Krua (*Butea superb* Roxb.) crude extract for all male production of Nile tilapia (*Oreochromis niloticuss*). *Journal of Agricultural Technology* 10.2: 391-398

Kotta, S., Ansari, S. H and Ali, J. 2013.Exploring scientifically proven herbal aphrodisiacs. *Pharmacognosy Review* 7.13: 1 – 10.

Kumoro, A. C. and Hartati, I. 2015. Microwave assisted extraction of dioscorin from gadung (*Dioscorea Hispida* Dennst) tuber flour. *Procedia Chemistry* 14: 47-55.

Kwikiriza, G., Barekye, A., Aheisibwe, A. R., Byakora, E. and Tibihika, P. D. 2017. Comparative growth performance and proximate nutrient composition of three local strains of Nile Tilapia (*Oreochromis niloticuss* L.) collected from different locations in Uganda. *Fisheries and Aquaculture Journal* 8.3: 5pp.

Lakshmi, G. D., Kumar, P.R. Bharavi, K. Annapurna, P. Rajendar, B. Patel, P. T. Satish-Kumar, C. S. V. and Roa, G. S. 2011. Protective effect of *Tribulus terrestris* Linn. on liver and kidney in cadmium intoxicated rats. *Indian Journal of Experimental Biology* 50: 141 – 146.

Lee, P. Y., Costumbrado, J., Hsu, C. Y. and Kim, Y. H. 2012. Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments* 62: e3923, doi:10.3791/3923.

Li, H., Deng, Z., Wu, T., Liu, R., Loewen, S. and Tsao, R. 2012. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chemistry* 130: 928–936

Lillehoj, H. Liu, Y., Calsamiglia,S., Fernandez-Miyakawa, M. F., Chi, F. Cravens, R. L., Oh, S. and Gay, C. G. 2018. Phytochemicals as antibiotic alternatives to

promote growth and enhance host health. *Veterinary Research* 49: 76 doi: 10.1186/s13567-018-0562-6

Madalla, N., Agbo, N. W. and Jauncey, K. 2016. Evaluation of ground - sundried cassava leaf meal as protein source for Nile Tilapia *Oreochromis niloticuss* (L) juvenile's diet. *Tanzania Journal of Agricultural Sciences* 15.1: 1 – 12.

Maheswari, M. S. U. and Rajendran, R. 2018. Comparative Evaluation of Qualitative and Quantitative Phytochemical Analysis of South Indian Bioactive Medicinal Plant *Tribulus terrestris.International Journal of Scientific Research in Biological Sciences* 5.6: 144 – 149.

Mahomoodally, M. F. 2013. Traditional medicines in Africa: An appraisal of ten potent african medicinal plants. *Evidence-Based Complementary and Alternative Medicine* 617459: 14p http://dx.doi.org/10.1155/2013/617459

Majekodunmi, S. O. 2015. Review of extraction of medicinal plants for pharmaceutical research. *Merit Research Journal of Medicine and Medical Sciences* 3.11: 521 – 527.

Makori, A. J., Abuom, P. O., Kapiyo, R., Anyona, D. N. and Dida, G. O. 2017. Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticuss*) growth in earthen ponds in Teso North Sub-County, Busia County. *Fisheries and Aquatic Science* 20.30: Accessed 10th August, 2019 from https://fas.biomedcentral.com/articles/

Manaf, S. R., Daud, H. H., Alimon, A. R., Mustapha, N. M., Hamdan, R. H., Muniandy, K. G., Mohamed, N. F., Razak, R. A. and Hamid, N. H. 2016. The effects of *Vitex trifolia*, *Strobilanthes crispus* and *Aloe vera* herbal-mixed dietary supplementation on growth performance and disease resistance in red hybrid tilapia (*Oreochromis* sp.). *Journal of Aquaculture Research and Development.* 7: 425. 5pp.

Manikandaselvi, S., Ravikumar, R., Thinagarbabu, R., Davidraj C. and Arvind, S. 2013. Hepatoprotective potential of *Azima tetracantha* and *Tribulus terrestris* on ferrous sulfate-induced toxicity in rat. *Bangladesh Journal of Pharmacology* 8: 357-360

Manish, K., Meenakshi, P. Ravindra, S. and Ashok, K. 2009. Evaluation of radiomodulatory influence of *Tribulus terrestris* root extract against gamma radiation: Hematological, biochemical and cytogenetic alterations in Swiss albino mice. *Pharmacology online* 1: 1214 – 1228.

Martínez, P., Viñas, A. M., Sánchez, L., Díaz, N., Ribas, L., and Piferrer, F. 2014. Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. *Frontiers in Genetics* 340.5: 1 – 13.

Mathur, M. and Sundaramoorthy, S. 2013. Ethnopharmacological studies of *Tribulus terrestris* (Linn). in relation to its aphrodisiac properties. *African Journal of Traditional, Complementary and Alternative Medicines*. 10.1: 83–94.

Mbaebie, B. O., Edeoga, H. O. and Afolayan, A. J. 2012. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine* 2.2: 118 – 124.

Megbowon, I. and Fashina-Bombatta, H. A. 2010. Tilapia: Fish for the Millennium. *Fish network* (Fisheries Society of Nigeria Quarterly Publication) 3: 36 – 40.

Megbowon I. and Mojekwu, T. O. 2014. Tilapia sex reversal using methyl testosterone (MT) and its effect on fish, man and environment. *Biotechnology* 13: 213-216.

Mekkawy, I. A. A., Mahmoud, U. M., Osman, A. G. and Sayed, A. E. H. 2010. Effects of ultraviolet A on the activity of two metabolic enzymes, DNA damage and lipid peroxidation during early developmental stages of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Fish Physiology and Biochemistry* 36: 605 – 626.

Mekkawy, I., Mahmoud, U. M. and Mohammed, R. H. 2013. Protective effects of tomato paste and vitamin E on atrazine-induced hematological and biochemical

characteristics of *Clarias gariepinus* (Burchell 1822). *Global Advanced Research Journal on Environmental Science and Toxicology* 2.1: 11 – 21

Miller, J. W. and Atanda, T. 2011. The rise of peri-urban aquaculture in Nigeria. *International Journal of Agricultural Sustainability* 9.1: 274 – 281.

Mjoun, K., Rosentrater, K. and Brown, M. L. 2010. Tilapia: Environmental biology and nutritional requirements. South Dakota Cooperative Extension Service. Retrieved Sept 30, 2011 from http://pubstorage.sdstate.edu/AgBio_Publications/articles/FS963-02.pdf. pp 7

Mlalila, M., Mahika, C., Kalombo, L., Swai, H. and Hilonga, A. 2015. Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. *Environmental Science and Pollution Research* Doi 10.1007/s11356-015-4133-3.

Mohamed, F. A., Khogali, F. A. Mohamed, A. H., Deng, O. O. and Mohammed, A. A. 2016. Body weight characteristics and chemical composition of Nile tilapia *Oreochromis niloticuss* collected from three different Sudanese dams. *International Journal of Fisheries and Aquatic Studies* 4.5: 507 – 510.

Mohammadi, M., Soltani, M., Siahpoosh, A., Shekarabi, S. P. H., Mehrgan, M. S. and Lymbery, A. 2018. Effect of date palm (*Phoenix dactylifera*) seed extract as a dietary supplementation on growth performance immunological haematological biochemical parameters of common carp. *Aquaculture Research* 49.8: 2903 – 2912.

Moradikor, N., Hajmohamadi, S. and Moradikor, Z. 2013. Physiological and pharmaceutical effects of *Tribulus terrestris* as a multipurpose and valuable medicinal plant. *International Journal of Advanced Biological and Biomedical Research* 1.10: 1289 – 1295.

Muanya, C. 2015. Scientifically proven herbal aphrodisiacs. *The Guardian.* Retrieved Mar 13th, 2019, from https://guardian.ng/sunday-magazine/living-wellbeing/scientifically-proven-herbal-aphrodisiacs/

Mubarik, M. S. 2011. The role of androgen on masculinization and growth performance of *Cyprinus carpio*; PhD thesis, University of Agriculture, Faisalabad Pakistan; xiii+13

Muir, J. 2013. Fish, feeds and food security. *Animal Frontiers* 3.1: 28–34.

Mukherjee, D., Ghosal, I. and Chakraborty, S. B. 2015a. Production of monosex Nile Tilapia, *Oreochromis niloticuss* using seed of *Mucuna pruriens. IOSR Journal of Pharmacy and Biological Sciences* 10: 55 – 59.

Mukherjee, D., Ghosal, I. and Chakraborty, S. B. 2015b. Application of *Asparagus racemosus* roots for production of monosex Nile tilapia, *Oreochromis niloticuss. International Journal of Advanced Research* 3.9: 828 – 833.

Mukherjee, D., Ghosal, I., Hanez, C. and Chakraborty, S. B. 2018. Dietary administration of plant extracts for production of monosex Tilapia: Searching a suitable alternative to synthetic steroids in Tilapia culture. *Turkish Journal of Fisheries and Aquatic Sciences* 18: 267 – 275.

Murov, S. 2018. Properties of solvents used in organic chemistry. Accessed 28 May, 2019 from http://murov.info/orgsolvents.htm

Murtha, J. M., Qi, W. and Keller, E. T. 2003. Hematologic and serum biochemical values for Zebrafish (*Danio rerio*). *Comparative Medicine* 6\53.1: 37 – 41.

Naima, R., Oumam, M., Hannache, H., Sesbou, A., Charrier, B., Pizzi, A. and Charrier-El Bouhtoury, F. 2015. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan *Acacia mollissima* barks. *Industrial Crops and Products* 70: 245-252.

National Bureau of Statistics (NBS) 2017. Nigeria's Fish Production (2010 - 2015) 22pp.

Ng, W.K. and Leger, M. 2013. Species profile: Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). In Aquaculture Feed and Fertilizer Resources Information System (www.fao.org/fishery/affris/en/). Rome, FAO. Retrieved 14th March 207

from http://www.fao.org/fishery/affris/species-profiles/nile-tilapia/nutritional-requirements/en/

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D. and Vuong, Q. V. 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the Root of *Salacia chinensis* L. *Journal of Food Quality* 9305047: 8pp 9305047: 8pp doi.org/10.1155/2017/9305047.

Ngueku, B. B. 2015. Cultivable fish species of river awe, Nasarawa state, Nigeria. *International Journal of Fauna and Biological Studies 2 (2): 15-19*

Nian, C. T., Tumbokon, B. L. M. and Serrano, A. E. 2017. *Pinus tabulaeformis* pollen as replacement for 17-alpha-methyltestosterone in the diet of *Oreochromis niloticuss* larvae for sex reversal and growth. *The Israeli Journal of Aquaculture - Bamidgeh, IJA_69.2017.1399* 9 p.

Nikolova, A. and Vassilev, A. 2011. A study on *Tribulus terrestris* L. anatomy and ecological adaptation. *Biotechnology and Biotechnological Equipment* 25.2: 2369 – 2372.

Nwachi, O. F. and Yuzine, B. E. 2016. A review of production protocols used in producing economically viable monosex Tilapia. *Journal of Fisheries and Aquatic Science* 11.1: 1 – 11.

Obadoni, B. O. and Ochuko, P. O. 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences* 8: 203 – 208.

Obaroh, I. O., Keta, J. N. and Bawa, D. Y. 2014. Haematological indices of *Oroechromis niloticuss* treated with Crude Extract *Mangifera indica* Leaf. *European Journal of Biotechnology and Bioscience* 2.5: 15 – 20.

Odin, R. Y., Germino, L. S., Noscal, L. D., Sugue, J. R. A., Argueza, R. L. B. and Abella, T. A. 2009. Masculinization of Nile tilapia (*Oreochromis niloticuss* L.) using testes from carabao (*Bubalus bubalis* L.), cattle (*Bos taurus* L.), and Hog (*Sus*

domesticus E.). In: C.C. Deocaris, H.M. Dejarme, A.M. Guidote Jr., L.P. Guidote, R.S. Julian and N.H. Tan Gana eds.. Book of Abstracts 29th Annual PAASE Meeting and Symposium Linking Science and Engineering to Development. The Philippine-American Academy of Science and Engineering PAASE). Quezon City, Philippines. Pp. 151.

Odufuwa, K. T., Atunnise, A., Kinnah, H. J., Adeniji, P. O. and Salau, B. A. 2013. Changes in saponins content of some selected Nigerian vegetables during blanching and juicing. *IOSR Journal of Environmental Science, Toxicology and Food Technology* 3.3: 38 – 42.

Okwodu, N. E. 2016. Aquaculture for sustainable development in Nigeria. *World Scientific News* 47.2: 151 – 163.

Olaoye, O. J. and Ojebiyi, W. G. 2018. Marine Fisheries in Nigeria: A Review. *Marine Ecology - Biotic and Abiotic Interactions*. M. Türkoğlu, U. Önal and A. Ismen. Eds. IntechOpen, DOI: 10.5772/intechopen.75032. Available from: https://www.intechopen.com/books/marine-ecology-biotic-and-abiotic-interactions/marine-fisheries-in-nigeria-a-review#B6. 155 – 173.

Olopade, O. A., Taïwo, I. O., Lamidi, A. A. and Awonaike, O. A. 2016. Proximate composition of Nile Tilapia (*Oreochromis niloticuss*) (Linnaeus, 1758) and Tilapia Hybrid (Red Tilapia) from Oyan Lake, Nigeria. *Bulletin UASVM Food Science and Technology* 73.1: 19 – 23.

Omar, E. A., Yousef, M. I, Srour, T. M. and Mansour, A. T. 2014. Effect of dietary natural phytochemicals on sex-reversal, growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticuss*) fry. *Spanish Journal of Advanced Agricultural Research* 19.3: 428 – 441.

Omitoyin, B. O. 2007. *Introduction to Fish Farming in Nigeria*. Ibadan University Press, University of Ibadan.105 pp.

Omitoyin, B. O., Ajani, E. K., and Sadiq, H. O. 2013. Preliminary investigation of *Tribulus terrestris* (Linn., 1753) extracts as natural sex reversal agent in

Oreochromis niloticuss (Linn., 1758) larvae. *International Journal of Aquaculture* 3.23: 133-137

Oner, M., Atli, G. and Canli, M. 2009. Effects of metal (Ag, Cd, Cr, Cu, Zn) exposures on some enzymatic and non-enzymatic indicators in the liver of *Oreochromis niloticuss*. *Bulletin of Environmental Contamination and Toxicology* 82.3: 317 – 321.

Ovie, S. I., Bwala, R. L. and Ajayi, O. 2011. A preliminary study on limnological stock assessment, productivity and potential fish yield of Omi Dam, Nigeria. *African Journal of Environmental Science and Technology* 5.11: 956 – 963.

Ozigbo, E., Anyadike, C., Adegbite, O. and Kolawole, P. 2014. Review of aquaculture production and management in Nigeria. *American Journal of Experimental Agriculture* 4.10: 1137 – 1151.

Pandey, A. and Tripathi, S. 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry* 2(5): 115-119.

Pandey, S. 2014. Preliminary Evaluation for Phytochemicals in *Tribulus Terrestris* and Its Antibacterial Activity. *Research and Reviews: Journal of Microbiology and Virology* 4.3: 6pp.

Patel, A., Soni, A. and Sharma P. 2016. In Vitro Cytotoxicity Studies of Indian Herb Tribulus Terrestris on Human Breast Cancer Cells. *IOSR Journal of Biotechnology and Biochemistry* 2.7: 84 – 90.

Patil, P. S., and Shettigar, R. 2010. An advancement of analytical techniques in herbal research *Journal of Advanced Scientific Research* 1.1:8 – 14.

Penkov, D. and Nikolova, M. 2016. Study on the effect of dry extract of *Tribulus terrestris* on the forage consumption rate in Japanese quail (*Coturnix coturnix japonica*). *Journal of Central European Agriculture* 17.1: 56 – 62.

Petenuci, M. E., Stevanato, F. B., Visentainer, J. E. L., Matsushita, M., Garcia, E. E., de Souza, N. E. and Visentainer, J. V. 2008. Fatty acid concentration, proximate composition and mineral composition in fishbone flour ofNile Tilapia. *Archivos Latino-americanos De Nutricion* 58.1: 87 – 90.

Petrovska, B. B. 2012. Historical review of medicinal plants' usage. *Pharmacognosy Review* 6.11: 1 – 5.

Pokrywka, A., Obmiński, Z., Malczewska-Lenczowska, J., Fijałek, Z., Turek-Lepa, E. and Grucza, R. 2014. Insights into supplements with *Tribulus terrestris* used by athletes. *Journal of Human Kinetics* 41: 99 – 105.

Prabu K, Samydurai P, Subbaiyan B, Thangapandian V. 2013. Phytochemical constitutents and gas chromatographymass spectrometry analysis of *Caralluma diffusa* (weight) N.E.BR. Aerial part. *International Journal of Pharmacy and Pharmaceutical Sciences* 5.3: 602 – 605.

Pradeep, P. J., Srijaya, T. C., Shaharom, F. and Chatterji, A. 2011. A brief of tilapia, their classification, distribution and perspective. In: F. Shaharom, P. J. Pradeep and C. H. Anil (Eds) Tilapia: Aquaculture techniques and potential, Universiti Malaysia Terengganu publication. 10pp.

Premarathna, A. D., Kumara., A. M. C. P., Jayasooriya., A. P., Satharasinghe, D. A. and Pathirana, E. 2018. Proximate analyses of Nile tilapia (Oreochromis niloticus) and Black tiger prawn (Penaeus monodon) from Sri Lanka. *Oceanography and Fisheries Open Access Journal* 7.5: 5pp.

Putra, A. A. S. Santoso, U., Lee, M. C. and Nan, F. H. 2013. Effects of dietary Katuk leaf extract on growth performance, feeding behavior and water quality of grouper *Epinephelus coioides*. *Aceh International Journal of Science and Technology* 2.1: 17 – 25.

Puttarak, P. and Panichayupakaranant, P. 2013. A new method for preparing pentacyclic triterpene rich Centella asiatica extracts. Nat Prod Res 27.7: 684-686

Rajaee A. H., 2011. Genetic Approaches to the Analysis of Body Colouration in Nile Tilapia (*Oreochromis niloticuss L*.) Ph.D thesis submitted to Institute of Aquaculture, University of Stirling Stirling, Scotland, UK. 190p.

Rakesh, P., Gyanendra, S., Anubhav, A. P. and Shubhini, A. 2009. Demand of herbal hepatoprotective formulations in Lucknow—a survey. *The Pharma Research* 01: 11pp.

Ramírez, E., López-Cardiel, J., Lezama, C., García-Márquez, L., Borja-Gómez, I and Tintos-Gómez, A. 2017. Effect of *Passiflora incarnata* (L) extract on gonadal maturation in young tilapia (*Oreochromis* sp.). *Latin American Journal of Aquatic Research* 45.5: 908 – 914.

Reitman, S. and Frankel, S. 1957. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *American Journal of Clinical Pathology* 28: 56-58.

Rezka, P., Balcerzak, W. and Kryłów, M. 2015. Occurrence of synthetic and natural estrogenic hormones in the aquatic environment. *Technical transactions Environmental engineering* DOI: 10.4467/2353737XCT.15.359.4824, 8p.

Roaiah, M. F., Khayat, Y. I. E., Din, S. F. G. and Salam, M. A. A. E. 2015. Pilot study on the effect of botanical medicine (*Tribulus terrestris*) on serum testosterone level and erectile function in aging males with partial androgen deficiency (PADAM). *Journal of Sex and Marital Therapy* 7:1-5.

Rodriguez-Fragoso, L., Reyes-Esparza, J., Burchiel, S. W., Herrera-Ruiz, D. and Torres, E. 2008. Risks and benefits of commonly used herbal medicines in Mexico. *Toxicology and Applied Pharmacology* 227.1: 125 – 135.

Şahin, A. and Duru, M. 2010. Effects of *Tribulus terrestris* (puncture vine) supplementation on performance and digestive system of broiler chicks. *Journal of Agricultural Science* 16: 271 – 277.

Saiyed, A., Jahan, N., Makbul, S. A. A., Ansari, M. Bano, H. and Habib, S. H. 2016. Effect of combination of *Withania somnifera* Dunal and *Tribulus terrestris* Linn on letrozole induced polycystic ovarian syndrome in rats. *Integrative Medicine Research* 5.4: 293 – 300.

Salman A. H. A. 2000. Effect of storage temperature on the quality and quantity of DNA extracted from blood. *Pakistan Journal of Biological Sciences* 3: 392 – 394.

Samani, N. B., Jokar, A., Soveid, M., Heydari, M. and Mosavat, S. H. 2016. Efficacy of the hydroalcoholic extract of *Tribulus terrestris*on the serum glucose and lipid profile of women with *Diabetes mellitus*. *Iranian Journal of Medical Sciences* 41.3: S5

Sansebastiano, G., Benedictis, M., Carati, D, Lofrumento, D, Durante, M., Montefusco, A., Zuccarello, V., Dalessandro, G. and Piro, G. 2013. Quality and efficacy of *Tribulus terrestris* as an ingredient fordermatological formulations. *The Open Dermatology Journal* 7: 1 – 7.

Santos, C. A., Reis, L.O., Destro-Saade, R., Luiza-Reis, A. and Fregonesi, A. 2014. *Tribulus terrestris* versus placebo in the treatment of erectile dysfunction: A prospective, randomized, double-blind study. *Actas Urológicas Españolas* 38.4: 244-248.

Satheeshkumar, P., Ananthan, G., Kumar, D. S. and Jagadeesan, L. 2011. Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. *Comparative Clinical Pathology* 21.6: 1 – 5.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. 2013. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 1.6: 168 – 182.

Sayed, A. H. and Moneeb, R. H. 2015. Hematological and biochemical characters of monosex tilapia (*Oreochromis niloticuss*, Linnaeus, 1758) cultivated using methyltestosterone. *The Journal of Basic and Applied Zoology* 72: 36 – 42.

Semerdjieva, I. B. and Zheljazkov, V. D. 2019. Chemical Constituents, Biological Properties and Uses of *Tribulus terrestris*: A Review. *Natural Product Communications* DOI: 10.1177/1934578X19868394 26pp.

Senanan, W. and Bart, A. N. 2010. The potential risks from farm escaped tilapias. Retrieved 17 September, 2019 from http://media.sustainablefish.org/Tilapia_escapes_WP.pdf

Sepperumal, U. and Saminathan, S. 2013. Effect of diethylphthalate on the haematological parameters of the freshwater fish *Oreochromis mossambicus* (Tilapia). *European Journal of Zoological Research* 2.4: 55 – 59.

Servati, H., Teli, A. S. and Zakeri, A. 2016. The effects of different levels of *Tribulus terrestris* extract on the performance and carcass characteristics of broiler chickens. *Advances in Bioresearch* 7.3: 171 – 177.

Shaheen, G., Ahmad, I., Usmanghani, K., Akhter, N., hmad M., Sabira S. and Akram, M. 2012. Monograph of *Tribulus terrestris*. *Journal of Medicinal Plants Research* 6.5: 641 – 644.

Shahid, M., Riaz, M., Talpur, M. M. and Pirzada, T. 2016. Phytopharmacology of *Tribulus terrestris. Journal of Biological Regulators and Homeostatic Agents* 30.3: 785 – 788.

Shamsuddin, M., Hossain, M. B., Rahman, M. M., Asadujjaman, M. and Ali, M. Y. 2012. Performance of monosex fry production of two Nile tilapia strains; GIFT and NEW GIPU. *World Journal of Fish and Marine Science* 4: 68-72

Sharma, M., Kumar, A., Akshita, B. S. and Dwivedi, N. 2013. Evaluation of phytochemical compounds and antimicrobial activity of leaves and fruits *Tribulus terrestris*. *European Journal of Experimental Biology* 3.5: 432 – 436.

Sheel, R., Nisha, K. and Kumar, J. 2014. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry* 7.1: 10 – 13.

Simon, C., Franke, A. and Martin, A. 1991. The Polymerase Chain Reaction: DNA Extraction and Amplification. In: G.M. Hewitt, A.W.B. Johnston, J.P.W. Young (Eds.) Molecular Techniques in Taxonomy. NATO ASI Series (Series H: Cell Biology), vol 57. Springer, Berlin, Heidelberg.

Singh, J. 2008.Maceration, Percolation and Infusion Techniques for the Extraction of Medicinal and Aromatic Plants. *Extraction Technologies for Medicinal and Aromatic Plants*.S. S. Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh. Eds. Italy: Padriciano 99, 34012 Trieste. Chapter 3: 67 – 82.

Singh, D., Abubakar, A. and Keta, J. N. 2010. Proximate composition of *Tribulus terrestris* L. leaves commonly found in some parts of Kebbi State, Nigeria. *Journal of Phytology Research* 23.2: 199 – 200.

Singh, S., Nair, V. and Gupta Y. K. 2012. Evaluation of the aphrodisiac activity of *Tribulus terrestris* Linn. in sexually sluggish male albino rats. *Journal of Pharmacology and Pharmacotherapeutics* 3.1: 43–47

Smith, S and Morin, S.A. 2005. Optimal storage conditions for highly dilute DNA samples: A role for Trehalose as a preserving agent. *Journal of Forensic Sciences* 50.5: 50 – 51.

Sofowora, A. E 1993. *Medicinal plants and traditional medicine in Africa*. 2nd Ed. Spectrum Book Ltd. Ibadan Nigeria. 405pp

Sogbesan, O. A. and Ekundayo, T. M. 2014. Cost benefits of fermented groundnut shell meal as supplemented feed in the diets of *Clarias gariepinus* fingerlings. *Nigerian Journal of Fisheries and Aquaculture* 2.2: 30 – 41

Sotolu, A. O., Kigbu, A. A. and Oshinowo, A. J. 2014. Supplementation of Date Palm (*Phoenix dactylifera*) Seed as Feed Additive in the Diets of Juvenile African Catfish (Burchell, 1822). *Journal of Fisheries and Aquatic Science* 9: 359-365.

Soto-Zarazúa, G. M., Rico-García, E., Ocampo, R., Guevara-Gonzalez, R. G. and Herrera-Ruiz, G. 2009. Fuzzy-logic-based feeder system for intensive tilapia production (*Oreochromis niloticuss*). *Aquaculture International* 18.3: 379 – 391.

Stadtlander, T., Levavi-Sivan, B., Kerem, Z., Dweik, H., Qutob, M., Abu-Lafi, S., Francis, G., Forken U. and Becker, K. 2012. Effects of a saponin fraction extracted from *Trigonella foenum-graecum* L. and two commercially available saponins on sex ratio and gonad histology of Nile tilapa fry, *Oreochromis niloticuss* (L.). *Journal of Applied Ichthyology*, 29: 265 – 267.

Su, L., Chen, G., Feng, S. G., Wang, W., Li, Z. F., Chen, H., Liu, Y. X. and Pei, Y. H. 2009. Steroidal saponins from *Tribulus terrestris*. *Steroids* 74: 399 – 403.

Sujatha, S. and Prakash, G. 2013. Bioactive screening and antimicrobial activity of flowers from the selected three medicinal plants on chosen microbes. *International Journal of Current Microbiology and Applied Sciences* 2.5: 211 – 221.

Sukmanomon, S., Kamonrat, W., Poompuang, S., Nguyen, T. T. T., Bartley, D. E., May, B. and Na-Nakorn, U. 2012. Genetic changes, intra- and inter-specific introgression in farmed Nile tilapia (*Oreochromis niloticuss*) in Thailand. *Aquaculture* 324-325: 44 – 54.

Sulaiman, S. F., Sajak, A. A. B., Ooi, K. L., Supriatno, S. E. M. 2011. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *Journal of Food Composition and Analysis* 24.4 – 5: 506 – 515.

Sultana, N., Noor, P., Abdullah, A. T. M., Hasan, M. R., Ahmed, K. M. and Naser, M. N. 2012. Growth Performance and Nutrient Composition of Juvenile Nile Tilapia (*Oreochromis niloticuss*) Treated with *Spirulina* Flakes, Rice Bran and Mustard Oil Cake. *Malaysian Journal of Nutrition* 18.2: 275 – 282.

Sun, Y., Jiang, D. N., Zeng, S., Hu, C. J., Ye, K., Yang, C., Yang, S. J., Li, M. H. and Wan, D. S. 2014. Screening and characterization of sex-linked DNA markers and

marker-assisted selection in the Nile tilapia (*Oreochromis niloticuss*) *Aquaculture* 433: 19 – 27.

Susmitha, S., Vidyamol, K. K., Ranganayaki, P. and Vijayaragavan, R. 2013. Phytochemical extraction and antimicrobial properties of *Azadirachta indica* (Neem). *Global Journal of Pharmacology* 7: 316-320.

Sveier, H., Raae, A.J. and Lied, E. 2000. Growth and protein turnover in Atlantic salmon (*Samo salar* L.); the effect of dietary protein level and protein particle size. *Aquaculture* 185: 101-120.

Svobodova, Z., Pravda, D. and Palackova, J. 1991. *Unified methods of haematological examination of fish*. Research Institute of Fish Culture and Hydrobiology, Vodnany, Czechoslovakia. 31pp.

Talasaz, A. H., Abbasi, M. R., Abkhiz, S. and Dashti-Khavidaki, S. 2010. Tribulus terrestris-induced severe nephrotoxicity in a young healthy male. *Nephrology Dialysis Transplant* 25.11: 3792 – 3793.

Tasgin, E., Lok, S., Demir, N. and Ozdemir, M. 2010. The Effect of Testosterone Used in Sportsmen on Routine Biochemical Parameters. *Journal of Animal and Veterinary Advances* 9: 2038 – 2040.

Tholkappiyan, B., Martin, A. C. and Narayanan, N. 2011. Quality control aspects of the whole plant caltrop. *International Journal of Pharmaceutical and Biological Sciences* 2.1: 593 – 600.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. 2011. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia* 1: 98-106.

Tonye, I. A. and Sikoki, F. D. 2014. Growth performance and proximate composition of *Oreochromis niloticuss* (Trewavas) treated with cobalt chloride incorporated diet. *Journal of Biology, Agriculture and Healthcare* 4.5: 106 – 125.

Trease, G. E. and Evans, W. C. 2002. Pharmacognosy. 15th Edn. Saunders, pp. 214-393.

Trinder, P. 1969. Determination of blood *glucose* using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology* 22.2: 158 – 161.

Turan, F. and Cek, S. 2007. Masculinization of African catfish (*Clarias gariepinus*) treated with Gokshura (*Tribulus terrestris*). *The Israeli Journal of Aquaculture - Bamidgeh* 59.4: 224-229.

Turan, F. 2017. The influence of licorice root (*Glycyrrhiza glabra*) on sex reversal in Guppy *Poecilia reticulata*. *International Journal of Secondary Metabolite* 4.3: 205-210.

Ugochukwu, S. C., Uche, I. A. and Ifeanyi, O. 2013. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Asian Journal of Plant Science and Research* 3.3: 10 – 13.

Uluköy, G., Kubilay, A., Didinen, B. I., Metin, S., Altun, S., Diler, Ö., Mammadov, R., Dulluç, A. 2018. Immunostimulant effects of geophyte plant extract on non-specific defense mechanisms of Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Limnology and Freshwater Fisheries Research* 4.1: 36 – 41.

Umadevi, V. and Srinathrao, 2017. *Tribulus terrestris* (Linn): botany, medicinal uses, chemical composition and in vitro regeneration a review. *European Journal of Pharmaceutical and Medical Research* 4.9: 324-330

United State Department of Agriculture (USDA), 2010. Agricultural Research Service, National Nutrition Data base for Standard reference. Release, 23. Nutrition Laboratory

Usman, H., Abdulrahman, F. I. and Ladan, A. A. 2007. Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris* L. (Zygophyllaceae) Growing in Nigeria. *Journal of Biological Sciences* 2.3: 244 – 247.

Valenzuela-Grijalva, N. V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Domínguez-Díaz, D. and González-Ríos, H. 2017. Dietary inclusion effects of phytochemicals as

growth promoters in animal production. *Journal of animal science and technology* 59.8: 17pp

Vasait R. D. 2017. Detection of phytochemical and pharmacological properties of crude extracts of *Tribulus terrestris* collected from tribal regions of Baglan (M.S.), India. *International Journal of Pharmacognosy and Phytochemical Research.* 9.4: 508 – 511.

Verma N., Kumar, A. Saggoo, M. I. S. Kaur, G. Singh, M. Kumar V. and Kumar, U. 2009. Phytochemical analysis and antimicrobial activity of crude extract of *Tribulus terrestris* fruits; a known medicinal weed. *Advances in Plant Sciences* 22.1: 155 – 158.

Vijayalakshmi, V., Krishnaveni, S. and Madhuramozhi, G. 2017. Toxic manifestations of mercuric chloride and polychlorinated biphenyls on the haematological aspects of *Danio rerioPharmaceutical and Biological Evaluations.* 4.1: 52-58

Vinatoru, M., Mason, T. J. and Calinescu, I. 2017. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. *Trends in Analytical Chemistry* 97:159–178.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y. and Gritsanapan, W. 2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Industrial Crops and Products* 44: 566-571.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., Ghaffar, R. and Ansad. 2013. Phytochemical analysis of medicinal plants occurring in Local Area of Mardan. *Biochemistry and Analytical Biochemistry* 2.4: 144. 4pp.

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S. S., Mungkung, R., Nawapakpilai, S. and Phillips, M.2014. Improving productivity and environmental performance of aquaculture. *Creating a Sustainable Food Future*1–60. http://pubs.iclarm.net/resource_centre/WRI-3729.pdf

Wei, S., Fukuhara, H., Chen, G., Kawada, C., Kurabayashi, A., Furihata, M., Inoue, K. and Shuin, T. 2014. Terrestrosin D, a steroidal saponin from *Tribulus terrestris* L., inhibits growth and angiogenesis of human prostate cancer in vitro and in vivo. *Pathobiology* 81: 123–132.

Witeska, M. 2013. Erythrocytes in teleost fishes: a review. *Zoology and Ecology* 23:4: 275 – 281.

Workagegn, K. B., Ababboa, E. D., Yimer, G. T. and Amare, T. A. 2014. Growth performance of the Nile Tilapia (*Oreochromis niloticuss* L.) treated with different types of diets formulated from varieties of feed ingredients. *Journal of Aquaculture Research and Development* 5.3: 4pp.

World Bank, 2013. Fish to 2030: prospects for fisheries and aquaculture. Agriculture and Environmental Services Discussion Paper 03, World Bank Report Number 83177-GLB http://documents.worldbank.org/curated/en/458631468152376668/pdf/

Wu, C. C., Liu, C. H., Chang, Y. P. and Hsieh, S. L. 2010. Effects of hot-water extract of *Toona sinensis* on immune response and resistance to *Aeromonas hydrophilain Oreochromis mossambicus*. *Fish and Shellfish Immunology*29: 258–263.

Wu, Q. S., Wang, C. M., Lu, J. J., Lin, L. G., Chen, P. and Zhang, Q. W. 2017. Simultaneous determination of six saponins in *Panacis Japonici Rhizoma* using quantitative analysis of multi-components with single-marker method. *Current Pharmaceutical Analysis* 13.3: 289–295.

World Wildlife Fund (WWF) 2016. Fishing for proteins: How marine fisheries impact on global food security up to 2050. A global prognosis. https://www.wwf.de/fileadmin/fm-wwf/Publikationen-PDF/WWF-Report_Fishing_for_Proteins_English_Version.pdf

Xiong, W., Chen, X., Lv, G., Hu, D., Zhao, J. and Li, S. 2016. Optimization of microwave-assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology. *Journal of Pharmaceutical Analysis* 6.6: 382 – 388.

Yeganeh, S., Sotoudeh, A. and Movaffagh, A. N. 2017. Effects of *Tribulus terrestris* extract on growth and reproductive performance of male convict cichlid (*Cichlasoma nigrofasciatum*). *Turkish Journal of Fisheries and Aquatic Sciences* 17: 1003 – 1007.

Yılmaz, E., Cek, S. and Mazlum, Y. 2009. The effects of combined phytoestrogen administration on growth performance, sex differentiation and body composition of sharptooth catfish *Clarias gariepinus* (Burchell, 1822). *Turkish Journal of Fisheries and Aquatic Sciences* 9: 33 – 37.

Yılmaz, E., Çek, Ş. and Mazlum, Y. 2013. Effects of synthetic and natural steroids on the growth, sex reversal and gonadal development of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fisheries and Aquatic Science* 30: 123 – 131.

Yingngam, B., Monschein, M. and Brantner, A. 2014. Ultrasound-assisted extraction of phenolic compounds from *Cratoxylum formosum* ssp. formosum leaves using central composite design and evaluation of its protective ability against H2O2 - induced cell death. *Asian Pacific Journal of Tropical Medicine* 7: S497-S505

Yosef, S. 2009. Farming the aquatic chicken: improve Tilapia in the Philippines. *Millions treated with: proven successes in agricultural development*. D. J. Spielman and R. Pandya-Lorch Eds. Washington DC: International Food Policy Research Institute 2009. Chapter 18: 125-130.

Younis, E. M., Abdel-Warith, A. A. and Al-Asgah, N. A. 2012. Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticuss* during short and long termsublethal exposure to zinc. *African Journal of Biotechnology* 11.19: 4442 – 4446.

Zebib, H. and Teame, T. 2017. Assessment of monthly physico-chemical properties and fish yields of two micro dams of Tigray Region, Northern Ethiopia. *International Journal of Fisheries and Aquaculture* 9.9: 92 – 97.

Zhang, Q., Wang, C. H., Ma, Y. M., Zhu, E. Y. and Wang, Z. T. 2013. UPLC-ESI/MS determination of 17 active constituents in 2 categorized formulas of traditional

Chinese medicine, Sanhuang Xiexin Tang and Fuzi Xiexin Tang: application in comparing the differences in decoctions and macerations. *Biomedical Chromatography* 27.8: 1079–1088.

Zhang, H., Wang, W., Fu, Z. M., Han, C. C. and Song, Y. 2014. Study on comparison of extracting fucoxanthin from *Undaria pinnatifida* with percolation extraction and refluxing methods. *Zhongguo Shipin Tianjiaji* 9: 91–95.

Zhang, Q. 2015. Effects of extraction solvents on phytochemicals and Antioxidant activities of walnut (*Juglans regia* l.) Green husk Extracts. *European Journal of Food Science and Technology* 3.5: 15-21

Zhang, Q. W., Lin, L. G., & Ye, W. C. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese medicine 13:* 20. 26pp.

Appendix

Plate 1.Harvested *T. terrestris* whole plant

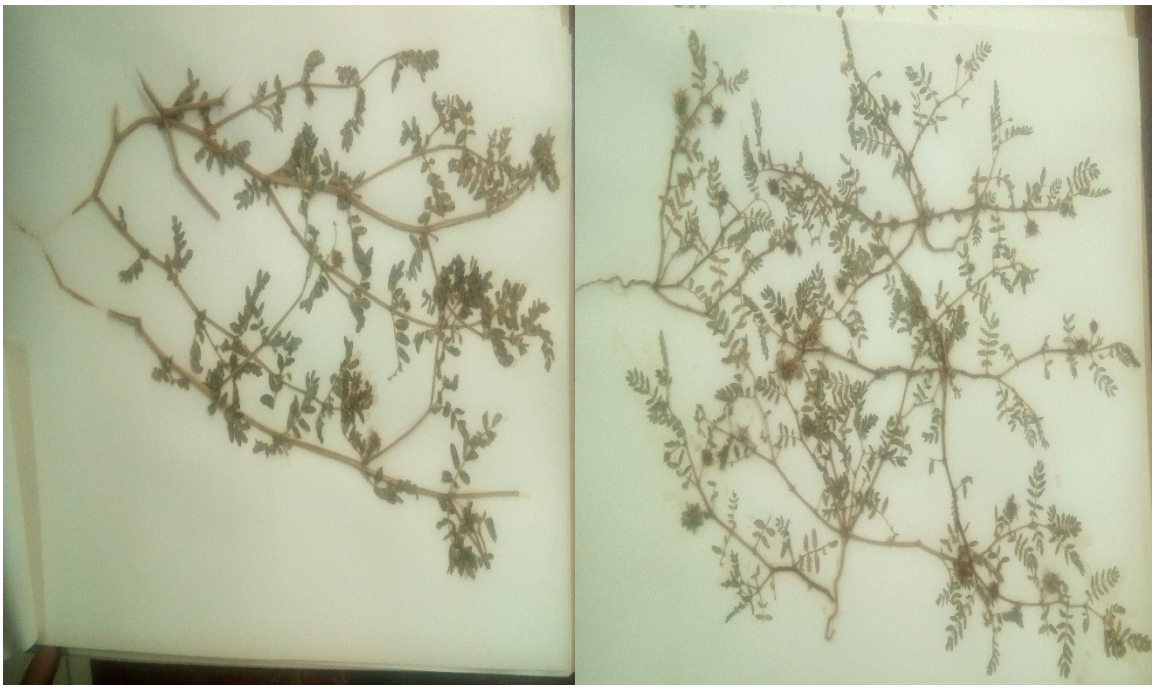

Plate 2. Sample of *T. terrestris* Deposited in the Herbarium