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Use of Herbal Extracts for Controlling Reproduction in Tilapia Culture: Trends and Prospects - a Review

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Abstract

The use of synthetic chemicals in the production of food for human consumption has been condemned by many nations, due to their potential health and environmental hazards. In tilapia farming in particular, synthetic sex reversal hormones have been commonly used to produce all male tilapia progenies. Recently, several herbal extracts have been reported to possess estrogenic properties, some of which are capable of inducing antifertility, abortifacient, and sex inversion in animals. Hence, herbal extracts could be used as safe alternative agents to control precocious tilapia maturity and prolific breeding in production systems. Dietary *Basella alba, Quillaja saponaria, Trigonella foenum-graecum, Glycine max* and *Tribulus terrestris* extracts have been reported to shift tilapia sex ratio in favor of males. Moreover, *Moringa oleifera, Carica papaya, Aloe vera, Azadirachta indica,* and *Hibiscus rosasinesis* extracts demonstrated a direct effect on gonad morphology and delayed maturation in some tilapia species. However, there are limitations, which make it hard for this novel development to progress from experimental trials to widespread adoption by farmers because of lack of adequate knowledge on phytoestrogen extraction methods, their extract concentrations, and identification methods. Hence, there is a need for more research to standardize every aspect concerning the use of phytoestrogens in tilapia culture. The aim of this paper is to provide an overview of the available studies on the use of herbal extracts as potential alternatives to control tilapia reproduction in aquaculture, while also discussing limitations in the existing knowledge and finding a way forward.

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Introduction

About half a decade ago, 14.3 % of the world's human population (6.7 billion) was declared undernourished, and the global population is expected to reach 9.3 billion by 2050 (UN, 2010). Agriculture, livestock and fisheries (inland and marine) which are the most important food producing sectors, are close to reaching their maximum capacity in terms of sustainable production (FAO, 2006). Thus the current food shortage is expected to be a long-term problem, unless major strides are taken towards meeting future demands for food. Aquaculture is considered one of the fastest growing animal food producing sectors globally, with a continuous annual growth rate of 8%, increasing production from 16.8 million tons in 1990 to 78.88 million tons in 2010 (FAO, 2012). Apart from being one of the few suitable options for feeding the growing global population with cheap and beneficial animal proteins, aquaculture is also regarded as a way of preserving wild fish stock, providing employment, and is an essential component of integrated rural development (Srinath et al., 2000).

In freshwater aquaculture, tilapia species are widely acknowledged as one of the most important internationally traded fish (FAO/GLOBEFISH, 2013), with a significant increase in production from 383,654 metric tons (mt) in 1990 to 3,500,000 mt in 2011 (Fitzsimmons, 2012) (Fig. 3). In addition to new intensive farming technologies, tilapia possess an impressive range of qualities that have made them very suitable for aquaculture in the 21st century. Their attributes include: their ability to reproduce easily in captivity; rapid growth, reaching their marketable size (in about six months); tolerance of a wide range of environmental conditions; resistance to stress and disease; occupying a low trophic level, with versatile feeding habits; acceptance of artificial feed immediately after yolk-sac absorption; adaptation to a variety of culture systems; and marketability: nutritious and palatable. (Teichert-Codding et al., 1992; Altun et al., 2006; Fitzsimmons et al., 2011; Ghosa and Chakraboty, 2014). Among tilapia aquaculture species, Nile tilapia (*Oreochromis niloticus*) has long been responsible for the significant increase in global production from freshwater aquaculture (FAO, 2002) (Fig.1),
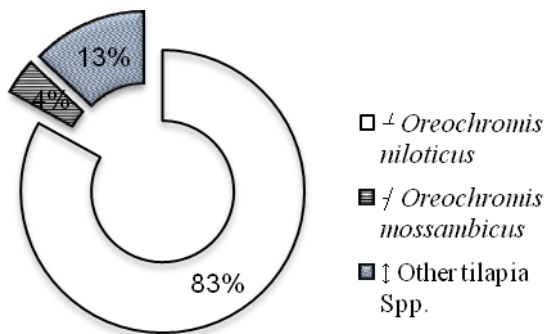
Fig. 1. Percent share of global tilapia aquaculture production by species (Data source; FAO, 2002).

This has been reported to account for a production rate above 3 million mt per year (Fitzsimmons et al., 2012) (Fig.3). On a global scale, China has long been the largest tilapia producer (Fitzsimmons et al., 2012; Tveteras, 2013), with production estimated at 1.3 million mt in 2011, about 40% of the global production (Tveteras, 2013) (Fig.2).
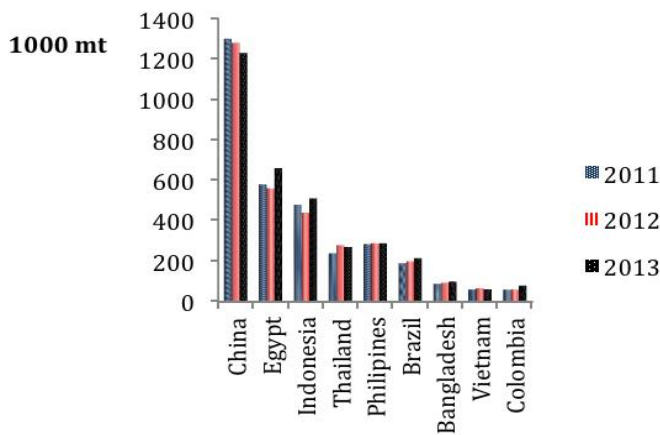
Fig. 2. Countries that significantly contributed to the world tilapia production from 2011- 2013 (adapted from Tveteras, 2013).

Regardless of the widely reported global tilapia aquaculture production and progress over the years, there are several challenges commonly associated with their reproductive ability that inhibit their full aquaculture potential. The most common setback in tilapia aquaculture is their precocious maturity and frequent breeding behavior (Mires, 1995).
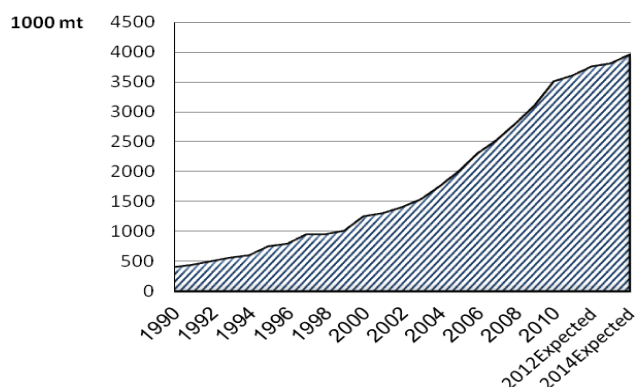
Fig. 3: Tilapia global aquaculture production from source; FAO, 2002).

Thus various techniques to control unwanted tilapia reproduction have been developed. Previous methods developed to control undesirable reproduction in tilapia production systems have led to the farming of all male progenies that grow faster and avoid the stunting of cultured populations (Guerrero, 1975; Mires, 1987; Jiménez-Badillo, 2006). An underlying reason for superior growth under these conditions is that they expend more energy on growth rather than on reproductive behavior (Phelps and Popma, 2000). Methods of producing all male tilapia include manual separation of sexes, hybridization, hormonal sex reversal, and genetic manipulation (Mires, 1977; Mair and Little, 1991), of which, the latter two methods are widely applied in commercial fish farming (Phelps and Poma, 2000; Wassermann and Afonson, 2002). To date, existing mono-sex production methods have suffered from limitations, rendering them ineffective, laborious, unsustainable, and to a large extent inaccessible to fish farmers, especially, rural small scale fish farmers (Hulata et al., 2004). It is important therefore that alternative methods of controlling precocious maturation and prolific breeding in tilapia culture and other aquaculture species be developed, to ensure a more cost effective and sustainable aquaculture industry.

Several studies have highlighted the benefits of medicinal herbs as potentially suitable alternatives to chemicals and drugs (Eckstein and Spira, 1965; Citarasu, 2010; Ganzera et al., 2001; Francis et al., 2002; Dabrowski et al., 2005; Dongmeza et al., 2006; Green and Kelly, 2009; Bai et al., 2012; Felicitta et al., 2013; Hu et al., 2014; Ghosal and Chakraborty, 2014; Gabriel et al., 2015). In addition to anti-stress, growth promotion, appetite stimulation, tonic and immune-stimulation, and antimicrobial properties (Citarasu, 2010; Chakraborty and Hancz, 2011; Ghosal and Chakraborty, 2014), medicinal herbs have also been reported to possess antifertility and abortificient properties when orally administered to animals (Obaroh and Chionye-Nzeth, 2011). Herbal compounds (phytoestrogens) such as isoflavonoids, flavonoids, lignans, and coumestans among others, are believed to mimic, or function as sex hormones, and are able to block biosynthesis and estrogen by acting as aromatase inhibitors and antagonists to nuclear estrogen receptors in gonad germ cells (Das et al., 2012), and may therefore potentially induce sex reversal, or to delay maturity in fish. The use of medicinal herbs at all levels of aquaculture may not only improve production, but could also enhance the safety and quality of aquatic products, thus increasing the use of these products across the globe.

The aim and scope of this article is: (1) to provide an overview of the available studies on the use of herbal extracts as potential alternatives in the control of tilapia reproduction in aquaculture, (2) to review the reproductive physiology of tilapia and factors affecting it, (3) to briefly discuss early-developed methods of producing monosex tilapia population in aquaculture, and (4) to increase knowledge and prospective use of herbal extracts as an alternative way to produce monosex tilapia in aquaculture.

Tilapia reproductive behaviors and physiology

The ability of all living organisms to reproduce (sexually or asexually) is a pre-requisite for their existence. Understanding reproductive behavior and physiology of animals may be important in the development of sustainable food production, conservation of biodiversity, habitat protection, and establishing restoration initiatives. Many studies have dealt with reproductive behavior and physiology of teleost fish. Besides eco-morphological characteristics, tilapia genera (*Oreochromis*, *Sarotherodon* and *Tilapia*) are

classified mainly based on their reproductive behavior (Trewavas, 1982). While some fish change sex during their lifetime, tilapia species are described as gonochoristics, where individuals sexually differentiate into males (pair testes) or females (pair ovaries) and remain the same sex throughout their life (Yamazaki, 1983; Nakumura et al., 1998). As part of their reproductive behavior, all tilapia species exhibit a high degree of parental care, seen in their nest building and substrate spawning nature (Pullin and McConnell, 1982; Trewavas, 1982). There are both differences and similarities in brooding of eggs and fry in tilapia species. *Oreochromis* and *Sarotherodon* species are both mouth brooders, meaning that eggs are fertilized in the nest, and then stored in their parents' mouth for incubation. The eggs are held in the mouth and the fry are held in the mouth for several days after hatching (Nandlal and Pickering, 2004). *Oreochromis* species possess a maternal mouth brooding nature, whereas, Sarotherodon species exercise either paternal or bi-parental mouth brooding behavior (Trewavas 1982; Nandlal and Pickering, 2004).

The easy and rapid propagation of tilapias in various tropical and sub-tropical environments make them ideal aquaculture species, however, this judgment is challenged by their reproductive efficiency combined with precocious maturation (as early as 3 months) (Pullin and McConnell, 1982; Phelps and Popma, 2000). In teleost fish and other vertebrates, reproduction is affected by many external factors such as temperature, photoperiod (Taranger et al., 2010; Bairwa et al., 2013), social patterns (Tubert et al., 2012; Maruska and Fernald, 2013) and internal factors, involving neuroendocrine system, (Schulz et al., 2010; Zohar et al., 2010; Bairwa et al., 2013).

The interaction between environmental factors, the neuroendocrine system, and reproduction in fish has not yet been fully explored, however, a considerable number of related studies have been published. Similar to other vertebrates, reproduction in fish depends on the neuroendocrine system, which initiates and controls gametogenesis (formation of female oocyte and male spermatozoon) and steroidogenesis (formation of steroids), through the activation of hypothalamus-pituitary-gonad axis (HPG) or the gonadotropic axis (Levavi-Sivan and Yaron, 1993; Kah et al., 1993; Van der Kraak et al., 1998; Fink, 2000; Tena-Sempere and Huhtaniemi, 2003; Kajimura et al., 2004; Marchesan et al., 2005; Pinilla et al., 2012). The initiation and completion of reproductive activity in teleost fish is controlled by photoperiod, which ensures an appropriate reproduction season favorable for offspring survival (Bromage et al., 2001). In addition, water temperature is believed to modulate HPG and affect the rate of gametogenesis or allow or inhibit gametogenesis to proceed beyond certain stages and/or completion (Mañaños et al., 1997; Prat et al., 1999). Furthermore, water temperature can affect the onset of reproduction or puberty indirectly in fish through its effect on somatic growth and energy storing (Taranger et al., 2010).

In tilapia species, the highest reproductive activity is associated with increasing photoperiod and warmer temperature, while low spawning rates are associated with lower temperatures and shorter photoperiod (Bairwa et al., 2013). One study concluded that a photoperiod of 12h light/dark cycle in Nile tilapia aquaculture ensured maximum fecundity, seed production, and spawning frequency (El-Sayed and Kawanna, 2007), although a minimum temperature range of $20-23^0$ is reported to be suitable for breeding in most tilapia species (Bairwa et al., 2013). Under subtropical and temperate conditions, where temperature or photoperiods are more variable, a well-defined breeding season needs to be determined for most tilapia species (Bromage et al., 2001; Bairwa et al., 2013).

Methods to control tilapia reproduction in production systems

Wild spawns in culture ponds of tilapia have led to precocious maturity and uncontrolled reproduction in farmed tilapia. This has necessitated the development of various methods to mitigate these behaviors.

The main, method used to control reproduction in tilapia is the culture of all-male tilapia, attained through manual separation of sexes, hybridization, hormone induced sex reversal, and genetic manipulation (androgenesis, gynogenesis, polyploidy and transgenesis) (Eckstein et al., 1965; Guerrero, 1975; Mires, 1977; Lovshin et al., 1990; Mair and Little, 1991; Fortes, 2005). Other methods include intermittent harvest, high stocking density culture, biological control, sterilization, culture in cages (Mair and Little,

1991) and using organic toxicants and/ or other chemicals (Fortes, 2005). Among the methods listed above, hormonal sex reversal has been widely adopted in aquaculture across the globe, and has become a reliable all-male tilapia production technique responsible for the global success of tilapia production over the years (Pandian and Sheela, 1995). Recently, this method has been combined with genetic manipulation (Acosta and Gupta, 2010). However, some of these methods have limitations when implemented outside experimental studies or developmental trials.

Manual separation of sexes

In addition to tilapia species being gonochoristic, they also possess sexual dimorphic characteristics, which make it easy to sort them into males and females. Manual separation of sexes as a method of obtaining all male/monosex tilapia populations is strictly based on separating males from females by visual inspection of external urogenital pores, often with the aid of dye applied (Fortes, 2005; Fuentes-Silva et al., 2013). The genital papilla of male is simple and smaller with two openings; the urogenital opening, where the milt and urine are excreted and the anus, for the discharge of fecal waste, whereas the female has a flatter and lager papilla with three openings; the anus, the urethra (for excretion of urine) and the oviduct, where the eggs pass through. In addition to sexual dimorphism, secondary sex characteristics can also be used to help differentiate sexes in tilapia, for instance, looking at the dorsal and anal fins which are pointed in males but rounded in the females (Chervinski and Rothbard, 1982).

  With these methods, sex separation is carried out before fish reach sexual maturity (Mair and Little, 1991), when they are large fingerlings (50- 80g), however the reliability of sexing depends on the skill of the workers, the species to be sorted, and its size (Fortes, 2005). Although this method is feasible, it is tedious, and difficult even for skilled workers to achieve 90 percent accuracy in sexing. Therefore, breeding and reproduction are rarely completely controlled (Mair and Little, 1991; Penman and McAndrew, 2000). Furthermore, this method may impact on economic returns of the fish farmer since in addition to the high cost of skilled laborers, about 40-50 percent of the female fingerlings are normally discarded (Fortes, 2005). Therefore this method is generally applied at subsistence level farming where fish populations are normally small; it may not be useful in commercial farming.

Hybridization

Hybridization, which is mating of genetically different individuals or groups, may involve crosses within a species (also known as line crossing or strain crossing) or crosses between species (Bartley et al., 2001). The rationale of this technique is to produce a hybrid or strain of superior quality than the parent species (Essa and Haroun, 1998). In aquaculture, hybridization is not only used to manipulate sex ratios or produce sterile fish, but also to increase growth rate, improve flesh quality, increase disease resistance, improve environmental tolerance, and improve a variety of other traits to make fish production more profitable (Bartley et al., 2001). Hybridization between tilapia species has attracted a lot of research (Table 1), which has subsequently led to the adoption of some tilapia hybrids (i.e. *Oreochromis aureus* x *O. mossambicus*; and *O. aureus* x *O. niloticus*) in commercial farms (Chapman, 1992).

  Despite the fact that hybridization is associated with the production of a high number of male progeny, this development is surrounded by numerous constraints, which make it unsustainable. Some of these constraints include: limited fecundity of parent fish which restrict fry production, difficulty in producing sufficient number of hybrid fry due to spawning incompatibility between parent species (Mires, 1977; Varadaraj and Pandian, 1989), and inasmuch as not all crosses produce 100% males, the hybrids may still require manual separation of sexes or hormone augmentation. In addition, widespread adoption of this method would result in the introgression of tilapia species with deleterious implications for the conservation of tilapia genetic resources (Mair and Little, 1991). More research is needed to either perfect this method or replace it with easily manageable and environmental friendly techniques.

Table 1**.** Hybridization of some tilapia species and proportion of male progeny produced

Cosses (♂ x ♀)	Males %	References
O. aureus x O. niloticus (Ugadan strain)	96-100	Pruginin, 1967;
O. aureus x O. vulcani	98-100	Pruginin, 1975;
O.hornorum x O. spilurus	100	Hulata et al. 1983
O. macrochir x O. niloticus	100	Wohlfarth et al., 1994
O.urolepsis hormorum x O. nigra	98-100	Wohlfarth et al., 1994
O.urolepsis hormorum x O. vulcani	98-100	Majumdar et al. 1983
O. macrochir x O. mossambucus	100	Majumdar et al., 1983
O.hornorum x O. niloticus	100	Wohlfarth et al., 1994
O.urolepsis hormorum x O. niloticus	100	Wohlfarth et al., 1990
O. aureus x O. niloticus (Stirling strain)	100	Marengoni et al., 1998
O.aureus x O. mossambicus	100	Beadmore et al,. 2001
O.hornorum x O. mossambicus	100	Hickling, 1960

Hormonal sex reversal
Constraints associated with manual separation of sexes and hybridization techniques have subsequently led to another technique called hormonal sex reversal. Hormonal sex reversal is the most efficient and commonly used method for mass production of all male tilapia in both small and large scale tilapia production (Pandian and Sheela, 1995; Phelps and Popma, 2000); and the success of global tilapia production is due to this technique.

Tilapia larvae are believed to be sexually undifferentiated up until 2 weeks after hatching, and at this time, larvae produce equal proportions of sex hormones; androgen (male), and estrogen (female) (Fuentes-Silva et al., 2013). Therefore, intervention or augmentation by exogenous steroid hormones such as androgen (male) or estrogen (female) during gonadal development or before sexual differentiation would influence the larvae to become either male or female depending on the hormone applied (Fortes, 2005; Fuentes-Silva et al., 2013). Two synthetic androgen hormones namely methyltestosterone (MT) and ethynyltestosterone have been widely used for masculinizing genotypic female tilapia (Mair and Little, 1991; Phelps and Popma, 2000; Forbes, 2005). Although, there is a wide range of hormonal administration methods, the commonly used treatment is oral administration for varying periods of 18-60 days in tanks or aquaria, with the dosage ranging from 10-60-mg/kg diet which is widely acknowledged as being effective for sex reversal (Guerrero, 1979).

Sex reversal success is achieved when uniform age fry or larvae eat only the hormone treated feed during the period of treatment, thus making it difficult to apply this technique in earthen ponds (Mires, 1995; Phelps and Popma, 2000). While many studies concluded that this technique offers a practical and economic approach for the control of tilapia reproduction (Guerrero, 1979), widespread use of large quantities of sex reversal hormones in hatcheries may pose a health risk to workers, consumers, and the environment (Phelps and Popma, 2000). In addition, hormones may be difficult to obtain in some countries, and hatchery facilities and skilled laborers are required (Forbes, 2005). Thus, this technology may create adverse effects in developing countries such as Sub-Sahara Africa, where aquaculture is still in the infancy stage, where there is poor infrastructure, poor or no protective equipment, and no effective guidelines on the use of hormones. More studies on the safe use of hormones in fish are crucial, for exploring affordable, environmentally friendly, and appropriate technology.

Genetic manipulation
The problems involved in the direct application of hormones to produce monosex (all-male tilapia) have eventually led to another alternative strategy in the production of an all-male tilapia population. Given the fact that tilapia species exhibit a predominantly monofactorial genotypic system similar to humans, male heterogamety (XY) and female homogamety (XX) (Penman et al., 1987; Mair et al., 1990; Mair and Little, 1991), a model was proposed for the production of monosex male progeny by genetic

manipulation of sex determination in *O. niloticus* (Mair and Little, 1991; Herrera and Cruz, 2001). This technique is based on the production of numbers of "supermale" of the novel genotype "YY'" which should yield all male progeny when crossed with normal females (Mair and Little, 1991). YY male technology was first shown in medaka, *Oryzias latipes* using the technology of hormonal sex reversal and selective breeding (Yamamoto, 1958). YY males were also produced in guppy, *Poecilia reticulata* (Yamamoto, 1963) in goldfish, *Carassius auratus* (Yamamoto, 1975), and then in tilapia, *O. niloticus* and *mossambicus* (Varadaraj and Pandian, 1989).

The procedures to produce YY supermale tilapia to yield all male tilapia (XY) have been well documented. The technology involves a series of stages of feminization and progeny testing (Herrera and Cruz, 2001). By adopting the Tuan et al. (1999) model to produce YY supermales, sex reversed females can be produced by oral application of a synthetic hormone, diethylstilbestrol (DES). The sex reversed females (XY) can be identified from DES-treated females by progeny testing with sex-reversed males (XX) of the same strain. Three confirmed sex-reversed females (XY) are then crossed with three normal males (XY) to generate YY supermales. The YY supermale is crossed with a normal female, which will eventually give all male progeny, called genetically generated male tilapia (GMT) (Figure 4). GMT and the YY supermales produced using this technology are not considered genetically modified organisms (Fortes, 2005). Compared to the hormonal sex reversal method, YY supermale technology is believed to be more feasible on a commercial scale, and is environmentally friendly since use of hormones on broodstock is limited and no hormonal residues are detected in consumed fish (Mair and Little, 1991). The genetic integrity of species or strain is also not affected, thus the fish produced for culture maintain a normal genotype (Trombka and Avtalion, 1993). Although GMT technology has been proven to be better than other tilapia monosex producing techniques (Mair et al., 1995), dissemination of information about its current application is limited especially in poor communities worldwide. In addition, the technique can be complex, time consuming, tedious, and still require sex hormones at its initial stage; it is only suitable for homogametic species (XX/XY) (Mair and Little, 1991).
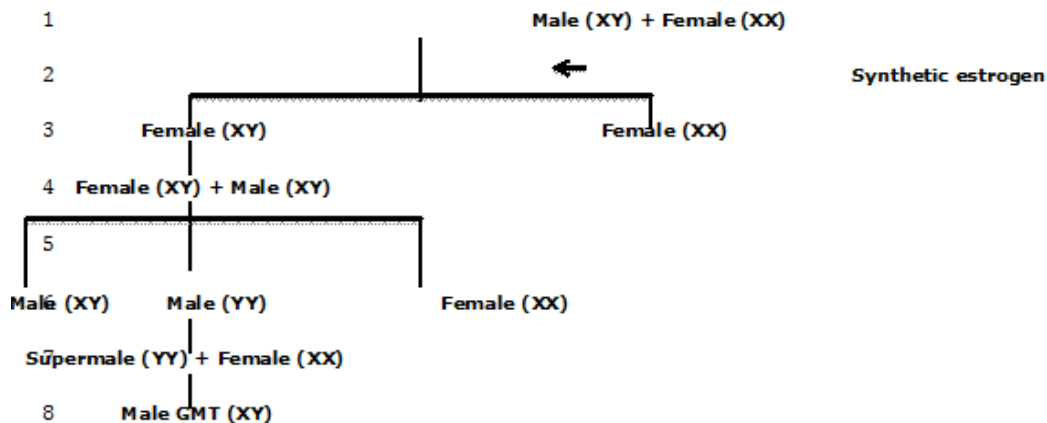
Fig.4: Schematic diagram for producing super male (YY) and all male tilapia (XY) (Tave, 1993).

Novel techniques to control tilapia reproduction
As discussed above, early methods of producing a monosex (all-male) tilapia population involved technical limitations that make these methods inappropriate for small aquaculture farms. The main concerns regarding these methods (especially synthetic sex hormone application) in tilapia production include; potential health risks caused by improper implementation of this system by farm workers, detrimental impacts on the environment, and social constraints (Mair and Little, 1991). To date, there is no substantial proof of any environmental damage or harm to humans caused by the synthetic hormone used for sex inversion, however the quantity of MT used in this practice is large compared to the actual dose required for sex reversal (Mlalila et al.,

2015), and this may lead to accumulation of this chemical in tilapia production systems.

This is in accordance with the previous study that reported that oral administration of MT to fish resulted in detectable levels of steroids in recirculating systems (Hulak et al., 2008). If this contaminated water is released into natural waters, this may have far-reaching effects of possible sex reversal in some natural populations, thus altering the sex dynamics of the stock, producing more males which ultimately may lead to recruitment failure (Megbowon, 2011). Therefore, the tilapia-farming sector is currently faced with a major challenge of finding sex control alternative methods, which are non-hazardous, cost effective, consumer and environmentally friendly. Studies have reported that water temperature and herbal extracts (phytochemicals) can influence hormone biosynthesis and the gonadal sex differentiation process (Baroiller et al., 2009; Bairwa et al., 2013; Fuentes-Silva et al., 2013), thus, they could provide alternative means of producing monosex fish populations in aquaculture, particularly in tilapia culture.

Temperature-induced monosex tilapia production

Tilapia is a thermo-sensitive species, its male to female ratio increases with temperature and/or ovarian differentiation induced by low temperatures (Fuentes–Silva et al., 2013). How does this happen? Studies have indicated that temperature is influential at a critical stage of sex differentiation in larval fish relatively similar to the hormone sensitive period. Inhibition of an enzyme called aromatase which catalyzes the conversion of androgen to estrogen during sex differentiation occurs, at high temperatures, thereby shifting larval or fry sex ratio to male (Brodie et al., 1999; Baroiller and D'Cotta, 2001). Masculinization of tilapia was possible at temperatures above 32°C (Baroiller et al., 1995). It has been suggested that this technique could be more effective when applied at least 10 days after fertilization. Sex determination in tilapia is governed by complex genetic interaction of exogenous factors including temperature (Baroiller et al. 2009). This method is novel therefore little of the mode of action is understood. Studies on temperature induced sex determination in tilapia are limited, thus, the mechanisms involved are yet to be established, given that, physiological, genetic or ecological studies cannot totally be depended upon, to better understand the dynamics of environmental sensitivity (Baroiller et al., 2009).

The use of temperature to produce monosex tilapia populations may be environmentally friendly and does not pose a health hazard to humans, however, at present, this method lacks sufficient control and complete sex reversal that is required to ensure its commercial application has yet to be established (Fuentes-Silva et al., 2013). Even if this method becomes effective in controlling tilapia reproduction, it is still not cost effective, and may be hard to apply to small-scale fish farming in poor rural areas. To the best of our knowledge there is no report to date of the commercial application of this technique in tilapia culture. Therefore, more economically viable tilapia biology and environmental research is needed.

Potential of Herbal extracts in producing monosex tilapia

The idea of using herbal extracts to produce monosex tilapia populations in aquaculture is novel, and operates on the principles of synthetic sex reversal hormones in fish culture. Several herbal extracts contain phytochemicals (phytoestrogens) which are structurally and/ or functionally similar to the steroid hormones i.e. 17-β estradiol (E2) in animals. They are capable of producing estrogenic effects in animals (Fowler, 1983; Lehtinen and Tana, 2001). The impaired effects of phytoestrogen were first discovered in the fertility of sheep and cattle that grazed on clover (*Trifolium subterraneum*) (Bennetts et al. 1946). These findings of could have set the pace for research on phytoestrogens, which subsequently led to the discovery of phytoestrogenic properties in several human foodstuffs including rice, soybeans, wheat, grain, potatoes, etc., and the isolation of two potential estrogenic substances from *Trifolium subterraneum (*Bradburry and White, 1954).

Furthermore, the reported potential of phytochemical/phytoestrogens to control reproduction in tilapia, have wide ranging consequences on various physiological processes in animals such as anti-stress, growth promotion, appetite, stimulation, tonic and immune-stimulation, and antimicrobial properties (Citarasu, 2010; Chakraborty and Hanz, 2011; Chakraborty et al., 2012). Nowadays, herbal extracts are preferred to synthetic drugs because they are cost effective, environmentally friendly, and less likely

to produce disease resistance due to their diversity (Logambal et al., 2000; Olusola et al., 2013). In humans, many women prefer phytoestrogens as alternatives to hormone replacement therapy (HRT) and estrogen replacement therapy (ERT), as they do not pose a risk of breast, endometrial cancer, or irregular bleeding (Brzezinski and Debi, 1999; Wade et al., 1999; Wagner et al., 2001).

Scientific databases such as Science Direct reported that the level of research and adoption of phytoestrogens in humans is more advanced than research in this area in aquaculture. Although the shift from synthetic drugs to herbal extracts has been increasing in aquaculture, more attention has been directed to the study of herbal extracts as growth, digestive and immune-stimulating enhancers in fish. Little research has been carried out on phytoestrogens as reproductive inhibitors in tilapia culture. Research which has paved the way to further studies on use of phytoestrogens in tilapia culture has investigated herbal extracts from; *Quillaja saponaria* (Angeles et al., 2015) and *Trigonella-foenum-graecum* (Francis et al., 2001; 2002; 2005; Stadtlander et al., 2008), *Azadirachta indica* (Jegede and Fagbenro, 2008), *Hibiscus rosa-sinesis* (Jegede, 2010), *Carica papaya* (Jegede, 2011; Abdelhak et al., 2013; Ampofo-Yeboah, 2013); *Aloe vera* (Jegede, 2011) *Moringa oleifera* (Ampofo-Yeboah, 2013), *Basella alba* (Ghosal and Chakraborty, 2014), Soybean (El-Sayed et al., 2012), and *Tribulus terrestris* (Omitoyin et al., 2013) (Table 2).

A study was conducted showing swollen spermatid nuclei, increased interstitial cells, and focal necrosis in testes, and hydropic degeneration, ruptured follicles, granulomatous inflammation in the interstitium, and necrotic ovaries when Neem leaves, *Azadirachta indica,* were incorporated in *Tilapia zilli* basal diet at 2.0 g/kg (Jegede and Fagbenro, 2008), (Table 3). Endocrine disrupting compounds (EDCs) including phytoestrogens may impair animal reproduction either by affecting gonad differentiation, by either directly affecting the gonad, or delaying maturation. Neem leaves may be an effective reproductive inhibitor in *Tilapia zilli*. Similar gonadal histological changes, due to incorporation of *Hibiscus rosa-sinesis* leaves at 3.0 g/kg in tilapia diet were reported (Jegede, 2010). *Aloe vera* latex was incorporated into a Nile tilapia diet at 2.0 mL/kg (Jegede, 2011) (Table 3). Similar findings were noted when pawpaw (*Carica papaya*) seeds were included in Nile tilapia basal diet at 120g/kg diets (Abdelhak et al., 2013) (Table 3). During these studies significant gonadal histological changes were reported at high doses, and a study confirmed that the effects (i.e. sterility) of papaya seed at high dose were permanent, while medium and low dose may have reversible effects (Abdelhak et al., 2013). These findings support the early studies, which reported that fertility rates in cheetahs in captivity were reduced when fed a feline diet of a soybean product. This effect was reversed when the soybean product was removed from the diet (Setchell et al*.*, 1987).

Furthermore, Saponin extracts from *Quillaja saponaria* (QS) (Francis et al., 2002), fenugreek (*Trigonella foenum-graecum*) and soapbark tree (*Quillaja saponaria*) (Stadtlander et al., 2008), and *Tribulus terrestris* (Omitoyin et al., 2013*),* reportedly shifted the normal 50:50 male to female sex ratio of Nile tilapia larvae in favor of males when incorporated in their diet, and a high percentage of males was recorded with high concentrations (Table 3). These masculinization effects of saponin extracts on tilapia larvae may be explained by the fact that saponin is able to elevate testosterone production (Ganzera et al., 2001), and as a result, plants that contain saponin compounds, particularly *Tribulus terrestris* have been used to treat impotence in humans (Adaikan et al., 2000; Gauthaman et al., 2002).

Table 2. Phytoestrogenic compounds and in vitro (human cells) status of some herbs.				
Family/ sub-family	Scientific / common name	Phytoestrogenic compounds	Phytoestrogenic activity in vitro	References
Liliaceae	*Aloe vera*	Flavonoids, Saponins, Anthraquinones	not confirmed	Patel et al. 2012
		Phenol, Steroids, Alkaloids		Kumar et al. 2012
	Aloe arborescens	not identified	confirmed	Matsuda et al. 2001
	Aloe forex	not identified	confirmed	Matsuda et al. 2001
	Aloe dracaena loureiroi	Chromanone	confirmed	Ichikawa et al.1997
Cannabaceae	*Humulus lupulus*	Flavone	confirmed	Hesse et al. 1981; Zava et al. 1998
				Milligan et al.1999
Moringaceae	*Moringa oleifera*	Flavonoids, phenol	not confirmed	Dillard and German, 2000
				Siddhuraja and Becker, 2003
Agavaceae	*Yucca sp.*	not identified	confirmed	Zava et al. 1998
Agavaceae	*Serenoa repens*	not identified	confirmed	Di Silverio et al. 1992
Cariceae	*Carica papaya*	Alkaloids, Flavonoids, Anthraquinone	not confirmed	Bamisaye et al. 2013
		Saponin, Phenol		
Poaceae	*Avena sativa* (oat)	Lignan	confirmed	Mazur, 1998
Annonaceae	*Annaxagorea luzonensis*	Prenylflavonoids	confirmed	Kitaoka et al. 1998
Euphorbiaeceae	*Jatropha curcas*	Alkaloids, Flavonoids, Saponin	not confirmed	Linda et al. 2014
		Phenol		
Meliaceae	*Azadirachta indica* (Neem)	Flavonoids, Phenol, Saponin, Alkaloids	not confirmed	Linda et al. 2014
Papavenaceae	*Sanguinaria Canadensis* (bloodroot)	Flavonoids, Phenol, Saponin, Alkaloids	confirmed	Zava et al. 1998
Rubiaceae	*Coffea arabica* (Coffee)	Lignan	confirmed	Kitts, 1987
	Uncaria tomentosa	Not identified	confirmed	Salazar and Jayme, 1998
Fabaceae	*Glycine max* (soy bean)	Lignan	confirmed	Rafi et al. 2000
Linaceae	*Linum usitatissimum* (Flax)	not identified	confirmed	Adlercreutz et al. 1992
Apiaceae	*Angelica sinensis* (dong quai)	not identified	confirmed	Dixon-Shanies and Shaikh, 1999
Verbenaceae	*Verbena officinalis*	not identified	confirmed	Dixon-Shanies and Shaikh, 1999
	Vitex agnus-castus	not identified	confirmed	Liu et al. 2001
Lamiaceae	*Leonurus cardiac*	not identified	confirmed	Zava et al. 1998
Basellaceae	*Basella alba*	Phenol, Flavonoids, Saponin	confirmed	Thirupathi and Rao 2014
Malvaceae	*Hibiscus macranthus*	Flavonoids, Tannin, Sterol	confirmed in bull leyding cells	Moundipa et al. 2005
	Hibiscus rosasinesis	Flavonoids, Tannin, Sterol	not confirmed	Soni et al. 2011
	Hibiscus sabdariffa Linn	Flavonoids, Tannin, Sterol	not confirmed	Kumar et al. 2012
Moraceae	*Maclcura pomifera*	Isoflavone	confirmed	Maier et al. 1995
	Morus microphyla	not identified	confirmed	Maier et al. 1995
Rosaceae	*Pranus africanum*	not identified	confirmed	Maier et al., 1995
Zingiberaceae	*Curcuma longa*	not identified	confirmed	Zava et al. 1998
Polygonaceae	*Fallopia multiflora*	anthraquinone	confirmed	Matsuda et al. 2001
	Rheum officinale	anthraquinone	confirmed	Matsuda et al. 2001

Contrary to what is expected of phytoestrogens in tilapia culture, soybean meal was reported to sharply reduce the percentage of males when added to Nile tilapia diet and tilapia farmers were cautioned to avoid the use of soybean as a source of protein during sex reversal treatment (El-Sayed et al., 2012) (Table 3).

The effects of phytoestrogens on animals depend on their ratio to endogenous estrogen, aromatase activity, animal species, reproduction status, length of exposure, and method of administration (Bennetau-Pelissero et al., 2001; Green and Kelly, 2009; Monteiro et al., 2000), and eventually, they may either exert the same effect as estrogen or block the effect of the estrogen (Andersen et al. 2003; Trant et al., 2001; Tsai et al., 2000). These facts therefore explain the estrogenic effects of soybean meal in Nile tilapia. We can see that phytoestrogens could be an alternative method to potentially control tilapia reproduction in production systems.

Table 3. In vivo herbal extracts inhibitory studies in tilapia reproduction

Scientific name	Extracts	Delivery	Concentrations	Exposure (days	Study	Effective doses	Tilapi spp.	References
Moringa oleifera	Seed powder	Oral	0.5, 1.0, 5.0, 10, 15g/kg	60	Gonad histology	5.0 g/kg	*O. mossambicus* fingerlings	Ampofo-Yeboa, 2013
	Seed powder	Oral	15 g/kg	90	Sex ratio	15 g/kg	*O. mossambicus* larvae	Ampofo-Yeboa, 2013
Canca papya	Seed powder	Oral	60, 90, 120 g/kg	60	Gonad histology	120 g/kg	*O. niloticus* fingerlings	Abdelhak et al. 2013
	Seed powder	Oral	0.5, 1.0, 5.0, 10, 15g/kg	60	Gonad histology	5.0 g/kg	*O. mossambicus* fingerlings	Ampofo-Yeboa, 2013
	Seed powder	Oral	15 g/kg	90	Sex ratio	15 g/kg	*O. mossambicus* fingerlings	Ampofo-Yeboa, 2013
Hibiscus rosasinesis	Leaves	Oral	1.0, 2.0, 3.0, 4.0 g/kg	60	Gonad histology	3.0 g/kg	*O. niloticus* fingerlings	Jegede, 2010
Azadirachta indica	Leaves	Oral	0.5, 1.0, 1.5, 2.0 g/kg	60	Gonad histology	2.0 g/kg	*T. zillii* fingerlings	Jegede & Fagbenro, 2008
Aloe vera	Latex	Oral	0.5, 1.0, 1.5, 2.0 mg/kg	60	Gonad histology	2.0 mg/kg	*O. niloticus* fingerlings	Jegede, 2011
Basella alba	Aqueous leaves	Immersion	0.05, 0.1, 0.15, g/l	30	Sex ratio	0.1 g/l	*O. niloticus* larvae	Ghosal & Charaborty, 2014
Ouillaja sapnonins	Saponin	Oral	50, 150, 300, 500, 700 mg/kg	60	Sex ratio	700 mg/kg	*O. niloticus* larvae	Francis et al. 2002
	Methanol saponin	Oral	40, 60, 80%, (150 & 1000ppm)	70	Sex ratio	80%, 150 ppm	*O. niloticus* larvae	Francis et al. 2002
T. foenum-fraecum	Methanol saponin	Oral	40, 60, 80%, (150 & 1000ppm)	70	Sex ratio	80%, 150 ppm	*O. niloticus* larvae	Statlander et al., 2008
Glycine max (soybean)	Genistein/Daidzein	Oral	10, 20, 30 mg/kg	28	Sex ratio	30 mg/kg	*O. niloticus* larvae	El Sayed et al. 2012
Tribulus terrestris Prododioscin	Saponin	Oral	1.0, 1.5, 2.0, 2.5 g/kg	42	Sex ratio	2.5 g/kg	*O. niloticus* larvae	Omitoyin et al, 20013

Phytoestrogens and their modes of actions

As discussed above, phytoestrogens are plant-derivatives that are structurally and /or functionally similar to mammalian estrogen 17β-estradiol (E2), therefore capable of producing estrogenic actions in animals (Price and Fenwick, 1985; Knight and Eden, 1996; Lehtinen and Tana, 2001; Ososki and Kennelly, 2003). The common groups of phytoestrogens include isoflavonoids (i.e. flavonols & isoflavans), coumestans (i.e. coumestrol), and lignan (Lehtinen and Tana, 2001). Among these groups, Isoflavonoids (e.g. genistein and daidzen) are the most well-known of the phytoestrogen and are by monocarboxylic derivatives of flavonoids with a carbon skeleton of 15 atoms similar to that of the coumestans (coumestrole) (Kaldas and Hughes, 1989) (Figure 5) whereas, the lignans have a skeleton based on 18 atoms (Adlercreutz, 1995). In addition to these groups of phytoestrogens, other classes of phytoestrogens include anthraquinones (Matsuda et al., 2001), Chalcones (Rafi et al., 2000), flavones (Milligan et al., 1999), prenylflavonoids, (Kitaoka et al., 1998) and saponins (Chan et al., 2002) (Table 2).

Isoflavonoids: Genistein

Isoflavonoids daidzen

Coumestans: Coumestrol

Lignan: Matairesinol

Anthraquinone: Aloin B

Saponins: Quillaja saponins
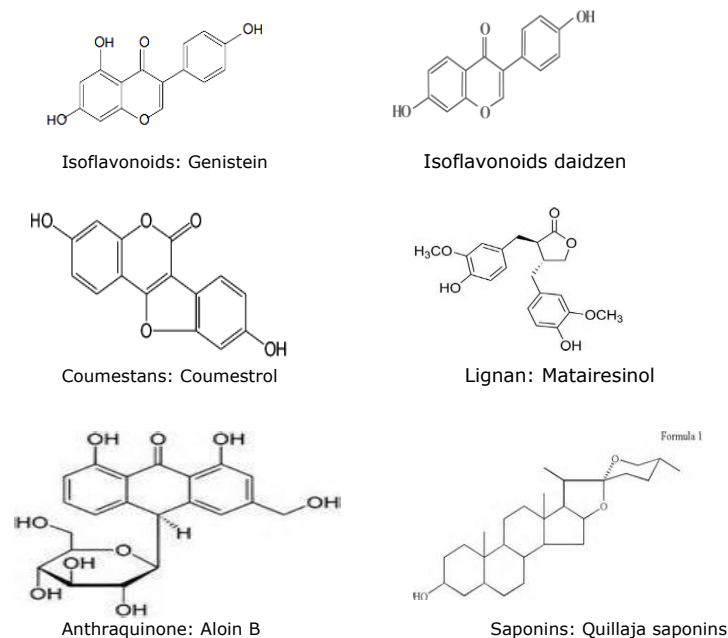
Fig 5. Structural formulas for some of the phytoestrogenic derivatives

The effects of phytoestrogens are partly based on the stability of their natural structures and low molecular weight, which allow them to pass through cell membranes and interact with enzymes and ERs to subsequently cause an estrogenic response (Adlercreutz, 1998), thus enhancing the success of masculinization in the sex inversion process of tilapia larvae. These effects may either be estrogenic or anti-estrogenic (Lehtinen and Tana, 2001; Ososki and Kennelly, 2003). Estrogenic potentials are able to mimic endogenous estrogens and cause estrogenic effects, whereas the anti-estrogenic potentials may block or alter estrogen receptors (ER) and prevent estrogenic activity, thus causing inverse estrogenic effects (Brzezinski and Debi, 1999); Ososki and Kennelly, 2003; Matozzo et

al., 2008). Phytoestrogens can be classified as selective estrogen receptor modulators (SERMs) (Brzezinski and Debi, 1999) with non-steroidal chemicals having similar structure to E2 and an affinity for estrogen receptors, and function either as agonists or antagonists (Gruber et al., 2002). Phytoestrogenic responses depend on several factors including methods of administration, dosage, metabolism of the target organism , intake of other chemical substances (Kelly et al., 1995; Xu et al., 1995), target tissues, number and types of ERs, and the presence or absence of endogenous estrogen (Glazier and Bowman, 2001).

There is insufficient knowledge on the mechanism of phytoestrogens in inducing estrus in animals. However, they are believed to exert estrogenic effects on the central nervous system, induce estrus, and stimulate growth of the genital tract in female animals (Ososki and Kennelly, 2003). In humans and rats, phytoestrogens are understood to bind to two ERs namely, ERβ and ERα, generally found in the brain, bladder, lungs, ovary, prostate, testis, uterus, spleen, and thymus tissues (Mosselman et al., 1996; Kuiper et al., 1997). In addition, a third ER called ERγ has been recently reported in different tissues of Atlantic croaker fish (Hawkins et al., 2000).

It has been observed that not all plants that show estrogenic activity can induce estrus; there are plant substances which are not phytoestrogens yet induce estrus (Ososki and Kennelly, 2003). This further illustrates the ambiguity in the mechanism of phytochemical inducement of estrus. Therefore, there is a need for more extensive research of phytoestrogens in a holistic manner.

Phytoestrogen screening and extraction techniques

There are a large number of techniques used to verify, quantify, and extract phytoestrogens from herbs and foodstuffs. Some of the quantification methods of phytoestrogens include bioassays, cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC), micellar electrokinetic capillary chromatography (MECC), nonaqueous capillary electrophoresis (NACE), gas chromatography coupled with a mass spectrometer (GC-MS), and high pressure liquid chromatography (HPLC) (Liang et al., 2009). As far as phytochemical assessments are concerned, these techniques have their own limitations. For instance, GC-MS as a quantification technique is reported to involve extensive purification procedures before analysis, thus making it more labor intensive and time consuming with much sample preparation (Wang et al., 2002), whereas, MEKC, MECC and NACE are associated with low reproducibility (Liang et al., 2009); bioassay techniques on the other hand have different level of sensitivity (Diel et al., 1999). Despite their limitations, these techniques are still useful, and are reported to be more effective when combined (Ososki and Kennelly, 2003; Ebuehi and Okorie, 2009).

Of these techniques, HPLC is the most commonly used method, since it involves limited sample preparation (Wang et al., 2002), and its usage is highly adaptable. Thus, it is most frequently combined with other techniques to improve phytochemical screening effectiveness. The combination of thin layer chromatography (TLC), infrared spectroscopy (IRS) and HPLC has been reported to be very effective to analyze and quantify flavonoids in *Jatropha curcas* leaves (Ebuehi and Okorie, 2009). In an attempt to quantify three phytoestrogens (Triterpene acid, Oleanolic acid, and Ursolic acid) in pawpaw and moringa seed powder respectively through ultra-high performance liquid chromatography combined with electrospray ionization mass spectrometer detection (UHPLC-ESI-MS/MS) (Ampofo-Yeboah, 2013), only Oleanolic acid in moringa could be quantified. Phytochemical screening is complicated, thus, it is always difficult to compare phytoestrogen content levels between herbs/plants and foodstuffs (Mazur, 1998).

In addition to the different phytoestrogen quantification techniques, there are also different kinds of phytoestrogen extraction methods such as solvents, solid phase extraction, and supercritical extraction (Asimi and Sahu, 2013). Among the three methods, solvents with different polarities such as petrol, ether, toluene, acetone, ethanol, ethyl acetate and water is the common phytochemical extraction method (Asimi and Sahu, 2013; Susmitha et al., 2013). A phytochemical screening study on Neem (*Azadirachta indica*) was able to extract flavonoids, alkaloids, steroids, saponin, and tanin in ethanol, methanol, acetone solution, respectively, whereas, water and ether were unable to extract steroids, saponins, and tanins (Susmitha et al., 2013). Meanwhile, Bamisaye et al. (2013) was able to extract all five phytoestrogens (flavonoids, alkaloids,

steroids, saponin, and tanin) extracted by Susmitha et al. (2013) from aqueous extracts of leaves and roots of *Carica papaya*.

Similar to phytoestrogens quantification techniques, combinations of solvents have been effective in extracting phytochemicals. For instance, a mixture of distilled water, ethanol, and acetone solution was reported to extract tannins, saponins, and flavonoids from *Aloe vera* leaves using GC-MS techniques (Arunkumar and Muthuselvam, 2009). Nevertheless, methanol/water/HCl (70:29: 1, v/v/v) mixture is reported to be the best of several solvent combinations used to extract phytochemicals (Asimi and Sahu, 2013). Therefore, the types of extraction solvents/techniques and phytochemical quantification/verification techniques remain very important aspects to consider in phytoestrogenic studies.

Administration of herbal extracts in aquaculture

In aquaculture, herbal extracts may be administered orally, by injection, and/or by immersion (Table 3). Injection and immersion may be the most effective methods of administering herbal extracts to fish, however, they may not be practical in aquaculture at all stages of production as they are expensive, labor intensive, and stressful to fish. Although oral administration is considered less effective, because absorption by fish is slow (Harikrishnan et al., 2009), this method allows a large number of fish to be treated with reduced stress and at lower cost, and labor input (Sakai, 1999), thus making it a potentially more suitable option in aquaculture. Herbal extracts incorporated in fish diets at different doses (Bulfon et al., 2013) depend on the extracts, size of the animals, farming system, and purpose of administration. There is a lack of standardization in herbal extract administration in aquaculture therefore more studies are needed to try and standardize methodology of extraction procedures, extract dosage, method of administration, and quantifying effects of herbal extracts in different aquaculture fish species.

Limitations in the existing knowledge and the path forward

Precocious maturity and prolific breeding of tilapia species in aquaculture presents numerous production challenges such as stunted populations, feed utilization, health and welfare. Consequently, the use of synthetic sex reversal hormones as a popular approach to mitigate this problem is more production oriented, while overlooking the impact of these hormones on the environment, and on humans, and animals. The idea that medicinal herbal extracts possess the ability to control tilapia reproduction in intensive production systems has been proposed [Francis et al. (2002; Jegede & Fagbenro 2008); Stadtlander et al. (2008); Jegede (2010; 2011); El- Sayed et al. (2012); Abdelhak et al. (2013); Ampofo-Yeboah (2013); Omitoyin et al. (2013)] however, this technology has yet to progress from experimental trials to widespread adoption by farmers. Compared to synthetic sex hormones, medicinal herbal extracts are easily accessible especially by small scale fish farmers, simple to apply, and may be safe for both the environment and humans as they tend to be more biodegradable (Logambal et al. 2000; Dabrowski et al., 2005; Olusola et al., 2013; Reverter et al., 2014).

The general application of herbal extracts in aquaculture is limited. The gap in existing knowledge limits the successful implementation of herbal extracts especially phytoestrogens. The problems include phytoestrogen identification methods, extraction methods, types of extracts (organic, aqueous, methanol, ethanol among others), harmful and beneficial doses for each aquaculture fish species, mode of application, mode of action, effects of different classes of phytoestrogens or herbal extracts in general on fish reproductive systems and other physiological processes such as energy metabolism, growth hormones and protein turnover. Saponin compounds found in most medicinal plants are believed to be promising tilapia masculinizing agents but reduce feed intake in fish (Dongmeza et al., 2006). Fish fed diets containing methanol extracted moringa leaf have been reported to show a better feed intake and growth performance than those fed with diets containing raw moringa leaf meal extract (Afuang et al., 2003). As stipulated above, methods of extraction and types of extracts are among the factors that determine the beneficial properties and efficacy of herbal extracts on the health of cultured fish. Several bioactive compounds are considered to be toxic (Dongmeza et al. 2006), and at present there is lack of knowledge on the toxicity of some herbal extracts in fish, consumers, and the environment. Furthermore, the effects of interaction of phytoestrogens and exogenous parameters including dietary inclusion levels are still

unknown. Therefore, the need to research more and standardize every aspect of the use of herbal extracts in aquaculture species is overwhelming. There is a need to explore and implement the use of herbs in a sustainable manner, as adopting them in aquaculture may double the pressure already exerted by agricultural sectors and humans.In conclusion, this review reveals that there are numerous medicinal plants with potential to control early maturity and prolific breeding in tilapia aquaculture. However, the lack of sufficient knowledge on herbal extracts limits the cost effective use of phytoestrogens in aquaculture. Therefore, more research is required to further validatethe use of herbal extracts with their allied phytoestrogenic activity, extraction methods, and extract concentration.

Acknowledgements

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