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Dietary *Aspilia mossambicensis* and *Azadirachta indica* supplementation alter gonadal characteristics and histology of juvenile Nile tilapia (*Oreochromis niloticus*)

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Abstract

The use of medicinal plants to control reproduction in aquaculture have recently received considerable attention because they are biodegradable, safe, effective and locally available. However, information on their effects on gonadal characteristics and histological features in fish is currently limited. The present study evaluated the gonadal characteristics and histological changes of Nile tilapia, *Oreochromis niloticus* gonads fed on Aspilia plant, *Aspilia mossambicensis* and Neem tree, *Azadirachta indica*. Juvenile fish were fed diets supplemented with four doses (1.0, 2.0, 4.0 and 8.0 g/kg diet) of *A. mossambicensis* or *A. indica* leaf powders at 3% of their body weight daily for 90 days to examine absolute fecundity (AF), relative fecundity, gonadosomatic index (GSI) and histology of gonads. The antifertility phytocompounds in the two plants were quantified by using standard methods. Results indicated that, all doses of *A. indica* and *A. mossambicensis* used reduced significantly AF and GSI values ($p < 0.05$). Dose-dependent histological alterations of gonads were noticed in both plants starting from doses of 2.0 g/kg in males and 4.0 g/kg in females. The highest percentages of total flavonoids (23.7%) and alkaloids (14.2%) were obtained from *A. indica* ethanol extract ($p < 0.05$). Taken together, dietary supplementation with *A. mossambicensis* and *A. indica* leaf powders alter gonadal characteristics of *O. niloticus* and histology triggered by alkaloids and flavonoids. Farmers interested in using the two plants to control prolific breeding should limit the dose inclusion to 2 g/kg to avoid severe effects on fish testis and ovaries.

KEYWORDS
antifertility, *Aspilia mossambicensis*, *Azadirachta indica*, histology, Nile tilapia, phytocompounds

1 | INTRODUCTION

Nile tilapia, *Oreochromis niloticus* is among the top ten farmed species in the world due to its hardiness, tolerance to varying degrees of physical and chemical environmental factors (Watanabe, Losordo, Fitzsimmons, & Hanley, 2002) among other attributes. Under natural conditions, *O. niloticus* starts to reproduce at a size of 20 to 30 cm (Lowe-McConnell, 1958). However when cultured in earthen ponds, it starts at a much smaller size of about 8 to 13 cm at which it undergoes successive reproductive cycles at intervals of three to six weeks (de Graaf, 2004). As a result, ponds become overcrowded

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with *O. niloticus* of varying sizes from multiple breeding, which results into competition for food and space and energy diversion from somatic growth to reproduction, ultimately affecting production (Coward & Bromage, 1999; Limbu et al., 2015). Thus, controlling prolific breeding is an important milestone towards higher production of *O. niloticus* in earthen ponds.

To curb the menace of prolific breeding, techniques such as polyculture of mixed sex *O. niloticus* with predatory fish (Limbu et al., 2015; Shoko, Limbu, Mrosso, & Mgaya, 2015), production of all males monosex (Dauda, Yakubu, & Oke, 2014; Mair, Abucay, Skibinski, Abella, & Beardmore, 1997) and culture in cages (Fortes, 2005; Mair & Little, 1991) have been researched and proposed. Production of all-male monosex using synthetic hormone is a much popular practice (Phelps & Popma, 2000). Nevertheless, fully application of synthetic hormone by fish farmers in developing countries is hampered by skills and unavailability (Limbu et al., 2015). Furthermore, synthetic hormones reduce fish immunity, survival and impose human and environmental health concerns (Abo-Al-Ela, 2018; Abo-Al-Ela, El-Nahas, Mahmoud, & Ibrahim, 2017). Therefore, alternative methods that are easy, safe, environmentally friendly and locally available to prevent prolific breeding of *O. niloticus* are needed.

Medicinal plants have long been recognized for their potential to regulate reproduction in humans and animals (Priya, Saravanan, & Renuka, 2012). Medicinal plants and their products are deemed appropriate because they are biodegradable, safe, effective and locally available to most fish farmers in developing countries (Kaur, Dhawan, & Shanthanagouda, 2017). However, the use of medicinal plants in aquaculture to control reproduction is a recent technology (Francis, Levavi-Sivan, Avitan, & Becker, 2002; Gabriel et al., 2017; Ghosal, Mukherjee, Hancz, & Chakraborty, 2015; Obaroh & Nzeh, 2013).

Among the different medicinal plants, *Aspilia mossambicensis* and *Azadirachta indica* have recently been utilized to control prolific breeding of *O. niloticus* (Kapinga, Limbu, Madalla, Kimaro, & Tamatamah, 2018). The *A. mossambicensis* is a medicinal plant which belongs to the genus *Aspilia* and is widely distributed in different parts of Africa (Norton, Huang, & Rodriguez, 1993). In Tanzania, the plant is found in areas along the Lake Victoria basin, Kigoma and Tanga (Smith, 1987). The plant is known to alleviate menstrual cramps and has been shown to induce uterine contraction and labour in pregnant women (Gruber & O'Brien, 2011; Musyimi, Ogur, & Muema, 2008). On the other hand, *A. indica* is a Meliaceae plant native to Burma, Nigeria, India and Pakistan, which can grow up to 30 m tall and 2.5 m girth (Ndodo et al., 2013; Silayo & Kiwango, 2010). In East Africa, the plant is famously known as the tree of the 40 due to its assumed ability to treat 40 different diseases (Silayo & Kiwango, 2010). Members of *Azadirachta* genus contain fertility regulating phytocompounds such as alkaloids and flavonoids (Susmitha, Vidyamol, Ranganayaki, & Vijayaragavan, 2013).

The ability of *A. mossambicensis* and *A. indica* to control prolific breeding of *O. niloticus* is due to the presence of phytocompounds. The phytocompounds in these plants are capable of interfering with normal synthesis, transport, metabolism and secretion of natural sex hormones or altering normal structures of reproductive organs (Kumar & Sachin, 2013). Recently we indicated that, *A. mossambicensis* and *A. indica* leaf powders modulate physiological parameters such as growth performance, feed utilization, haematology and control reproduction performance by reducing hatchlings production in *O. niloticus* (Kapinga et al., 2018). However, information on the effects of *A. mossambicensis* and *A. indica* leaf powders on gonadal characteristics and histological features in *O. niloticus* is currently limited.

Therefore, this study assessed the effects of *A. mossambicensis* and *A. indica* leaf powders on gonadal characteristics and histology of *O. niloticus*. In addition, phytochemical screening of *A. mossambicensis* and *A. indica* leaf powders was performed to quantify antifertility phytocompounds. The study hypothesized that, leaf powders from the two plants would affect gonadal characteristics and induce histological changes of *O. niloticus*.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The study was carried out in accordance with the Tanzanian laws and Sokoine University of Agriculture (SUA) guidelines for the care of experimental animals. All procedures of the current work were approved by the Committee of the College of Agriculture of the SUA.

2.2 | Plants collection and preparation

Based on ethno-botanical knowledge using available literature and visual observations, the plants were identified by a botanist. The leaves of *A. mossambicensis* were collected from Magamba village, Tanga region whereas *A. indica* were obtained from SUA main campus located in Morogoro Municipality. The plant leaves were thoroughly washed and then shade dried for two weeks in a ventilated room. Dry leaves were ground into fine powders by using a Lab Mill (Serial number 19,911, Christy Hunt Engineering, LTD, UK) fitted with a 1.0 mm screen and finally the powders were kept in dry containers and stored at room temperature until needed for use.

2.3 | Phytochemical analysis

The phytocompounds were analysed by using hot ethanol and cold water extractions according to standard procedures (Laghari, Memon, Nelofar, & Laghari, 2011; Novelli, Lorena, & Antonella, 2014). Total alkaloids were determined by spectrophotometric method as described by John, Sulaiman, George, and Reddy (2014) whereas total flavonoids were evaluated by methods described by Jing, Mohamed, Rahmat, and Abu Bakar (2010) and Pękal and Pyrzynska (2014). The data obtained were used to create a calibration curve (Supproting Information Figure S1). The content of total alkaloids and flavonoids were expressed as percentage of caffeine and catechin equivalent, respectively, per gram of dry powder of *A. indica* and *A. mossambicensis* leaves.

2.4 | Experimental diets preparation

The control diet (250 crude protein g/kg) was formulated according to Pearson's square by including fishmeal (sardines) and maize bran. The proximate compositions of the control diet and plants used in the present study are given in Table 1. The eight experimental diets were formulated by adding 1.0, 2.0, 4.0 and 8.0 g of either *A. mossambicensis* (AM1, AM2, AM4 and AM8) or *A. indica* (AI1, AI2, AI4, and AI8) to a kilogram of the control diet as we described previously (Kapinga et al., 2018).

2.5 | Experimental fish and their management

Experimental fish were collected from SUA ponds and transported to experimental tanks according to standard procedures recommended by Collart (1997). Thereafter, they were acclimatized to experimental conditions for two weeks. After acclimatization, twenty *O. niloticus* juveniles (10 females and 10 males per each tank) with mean weight of 41.5 ± 3.1 g and an age of four months were stocked in triplicates into 3.6 m^3 rectangular tanks supplied with 2,700 L water. Experimental fish were hand-fed twice daily at 10.00 and 17.00 hr at 3% of their body weight per day for 90 days. At the end of experiment, gonadal characteristics and histology were examined.

The water quality parameters in the experimental tanks were maintained at optimum recommended ranges for survival and growth of *O. niloticus*. Dissolved oxygen ranged from 6.0 to 7.6 mg/L, temperature from 26.7°C to 27.2°C and pH from 8.0 to 8.4. Complete water replacement for each tank was performed once a week.

2.6 | Data collection

After 3 months of feeding experiment, six juvenile fish (three females and three males per treatment) were weighed to obtain total wet weight. Thereafter, the fish were euthanized by immersing them in an ice slurry and dissected to obtain gonads (testis and ovaries). The weight of individual fish and their gonads were used to calculate gonadosomatic index (GSI) based on de Graaf (2004). The absolute fecundity (AF) was determined by counting the number of eggs in the ripe gonads through a gravimetric subsampling method (Bagenal, 1978), whereby a small portion of eggs from each lobe of the ovary was taken, weighed and the number of ripe eggs counted. The GSI, AF and RF were calculated by using the following formulae adopted from de Graaf (2004):

$$\text{Gonadosomatic index, GSI} = \left(\frac{\text{Weight of ovary (g)}}{\text{Individual fish body weight (g)}}\right) \text{x } 100$$

$$\text{Absolute fecundity, AF} = \frac{\text{Number of eggs in a sample (n) x weight of ovary (g)}}{\text{Weight of eggs (g)}}$$

$$\text{Relative fecundity, RF} = \frac{\text{Absolute fecundity (AF)}}{\text{Individual fish body weight (g)}}$$

TABLE 1 Proximate composition of control diet, *Aspilia mossambicensis* and *Azadirachta indica* (g/kg)

Composition	Control diet	*Aspilia mossambicensis*	*Azadirachta indica*
Moisture	88	91	83
Crude protein	250	216	147
Crude fat	101	24	15
Crude fiber	85	196	176
Ash	78	204	110
Carbohydrate	398	269	469

2.7 | Histological features of gonads

Fish gonads from six juvenile fish (three females and three males per treatment) were fixed by using 10% buffered formalin. After forty-eight (48) hrs, samples were dehydrated in a series of ethanol of varying concentrations from 70%, 90%, 95% and finally absolute ethanol (100%). Thereafter, specimens were thoroughly infiltrated into wax (waxing). Sections (5 μm) were made using Historange microtome (Laboratoire Kastler Brossel (LKB) Bromma 2,218), then stained by using hematoxylin-eosin (H & E). The stained sections were finally examined under light a microscope (Olympus BH-2) and photographed using a mounted digital camera.

2.8 | Statistical analyses

Results are presented as means ± *SD*. The data were first tested for normality and homogeneity of variances using Kolmogorov–Sminorv and Levene's tests, respectively. Thereafter, one-way analysis of variance (ANOVA) was used to test for significant differences in gonadal characteristics indices measured among the different diets. Tukey post hoc test was performed to determine specific significant differences among treatments. The percentage of alkaloids and flavonoids obtained from *A. indica* and *A. mossambicensis* using the ethanol and water extracts were compared by using Mann–Whitney test. Furthermore, the relationship between gonadal characteristics and plants doses was determined by using Pearson correlation. All statistical analyses were performed by using Statistical Package for the Social Sciences (SPSS) version 20 (IBM, Armonk, NY, USA) for Windows. Results with $p \leq 0.05$ were considered statistically significant.

3 | RESULTS

3.1 | Total alkaloids and flavonoids levels in the two plants

Results for phytochemistry were calculated according to calibration curve for caffeine (R^2 = 0.965) and catechin (R^2 = 0.997) for total alkaloids and flavonoids, respectively (Supproting Information Figure S1). Significantly higher percentage of total flavonoids (23.7%) in the hot ethanol extraction and alkaloids (14.2%) obtained in the cold

water extraction were found in *A. indica* compared to *A. mossambicensis* (Table 2; $p < 0.05$). Total alkaloids and flavonoids of water extracts for the two plants were not significant different ($p > 0.05$), yet values were relatively higher in *A. indica* compared to *A. mossambicensis*.

3.2 | Gonadal characteristics

The use of *A. mossambicensis* and *A. indica* leaf powders reduced significantly AF and RF of *O. niloticus* when compared to the control diet ($p < 0.05$; Table 3). The *O. niloticus* fed on *A. indica* at the higher dose (8.0 g/kg) had significantly lower AF and RF ($p < 0.05$) than those at the lower dose (1.0 g/kg). No significant differences were obtained in AF and RF between *O. niloticus* fed the lower and higher doses of *A. mossambicensis* leaf powder ($p > 0.05$). The lowest values of AF (343.3) and RF (3.8) were obtained in *O. niloticus* fed 4.0 g/kg *A. indica* and 1.0 g/kg *A. mossambicensis*, respectively. No significant change in GSI was obtained in *O. niloticus* fed on the two plants ($p > 0.05$). However, females fed the diets supplemented with the two plants had comparatively higher GSI values (>3.7) than males (<2.4). The GSI values obtained from *O. niloticus* fed on *A. indica* were relatively lower when compared to those fed on *A. mossambicensis* doses. Values of AF and RF decreased negatively as the doses increased for both *A. mossambicensis* and *A. indica* ($p < 0.05$).

3.3 | Histological features of testis and ovaries

In the control group, testes were covered by testicular capsule composed of connective tissues and a few blood vessels (Figure 1a). Testicular parenchyma was formed by seminiferous tubules lined by simple columnar and/or cuboidal cells. The lumen of seminiferous tubules was occupied by spermatozoa. No morphological changes were observed in the testis of *O. niloticus* fed on either *A. mossambicensis* or *A. indica* leaf powders at a dose of 1.0 g/kg. However, at a dose of 2.0 g/kg for both *A. mossambicensis* and *A. indica*, degenerating seminiferous tubules were separated by thick connective tissues (Figure 1b and c). Degenerating cells contained cytoplasmic pallor and fragmented nucleus and numerous vacuoles (Figure 1c insert). The lumen of seminiferous tubules of fish was devoid of spermatozoa whereas some contained spermatozoa intermixed with aggregates of inflammatory cells. At a dose of 4.0 g/kg for both *A. mossambicensis* and *A. indica*, degenerating seminiferous tubules were common in fish (Figure 1d and e). The lumen of seminiferous tubules was dilated and contained very few spermatozoa and inflammatory cells were observed scattered in the testicular parenchyma. At a dose of 8.0 g/kg for both *A. mossambicensis* and *A. indica*, the testicular lost its architecture, the thickness of connective tissue separating the seminiferous tubules increased and tufts of spermatozoa were observed in the degenerating seminiferous tubules in fish testis (Figure 1f and g). Accordingly, seminiferous tubules were lined by squamous cells and contained very few spermatozoa whereas inflammatory cells were observed between the tubules.

TABLE 2 Phytochemical composition of *Aspilia mossambicensis and Azadirachta indica* leaf powders

	Hot ethanol extraction (% g^{-1} dry powder)		Cold water extraction (% g^{-1} dry powder)	
Plant	Alkaloids (% g^{-1})	Flavonoids (% g^{-1})	Alkaloids (% g^{-1})	Flavonoids (% g^{-1})
AM	7.1 ± 3.1[a]	20.0 ± 3.0[a]	13.8 ± 0.1[a]	10.1 ± 3.8[a]
AI	12.5 ± 1.9[b]	23.7 ± 3.8[b]	14.2 ± 0.3[a]	19.0 ± 8.4[a]

Note. Values in the same column sharing the same superscript are not significant different ($p > 0.05$), AM = *Aspilia mossambicensis*, AI = *Azadirachta indica*.

TABLE 3 Gonadal characteristics of *Oreochromis niloticus* fed *Aspilia mossambicensis* and *Azadirachta indica* leaf doses

	Fecundity		Gonadosomatic index	
Treatment	Absolute	Relative	Males	Females
Control	698.3 ± 102.8[a]	10.7 ± 1.5[a]	2.5 ± 0.7[a]	3.4 ± 1.3[a]
AM1	361.0 ± 22.9[c]	3.8 ± 0.3[b]	1.6 ± 2.1 [a]	4.5 ± 2.9 [a]
AI1	459.0 ± 35.6[b]	7.4 ± 0.9[c]	0.9 ± 0.1[a]	4.3 ± 0.6[a]
AM2	444.3 ± 43.7[b]	4.7 ± 0.8[bd]	1.6 ± 0.6[a]	3.7 ± 0.5[a]
AI2	346.7 ± 34.5[c]	5.6 ± 0.3[d]	1.1 ± 0.3[a]	3.9 ± 0.5[a]
AM4	372.3 ± 94.5[bc]	4.8 ± 0.4[bd]	2.3 ± 0.7[a]	5.8 ± 2.5[a]
AI4	343.3 ± 33.5[c]	6.6 ± 1.5[cd]	1.1 ± 0.4[a]	3.8 ± 1.3[a]
AM8	383.3 ± 160.7[bc]	4.5 ± 1.9[bd]	2.1 ± 1.4[a]	4.7 ± 0.5[a]
AI8	364.0 ± 24.8[c]	4.7 ± 1.1[bd]	1.3 ± 0.1[a]	4.5 ± 0.2[a]

Note. Values in the same column sharing the same superscript are not significant different ($p < 0.05$). AM = *Aspilia mossambicensis*, AI = *Azadirachta indica*. AM1/AI1 = 1.0 g/kg, AM2/AI2 = 2.0 g/kg AM4/AI4 = 4.0 g/kg, AM/AI8 = 8.0 g/kg).

The ovary of control fish contained healthy follicles at different stages of development i.e. primordial, previtellogenic and vitellogenic follicles (Figure 2a). In vitellogenic follicles, the oocyte contained ooplasmic vesicles filed with vitellogenic globules, few vacuoles and lipid droplets were observed on the peripheral regions. Single layer of granulosa cells surrounded the ooplasm, the cells contained single nucleus located centrally and thecal layer was clearly separated from theca interna and theca externa. No histological changes were observed in the ovarian follicles of *O. niloticus* at doses of 1.0 and 2.0 g/kg for both *A. mossambicensis* and *A. indica*. However, at doses of 4.0 g/kg (Figure 2b and c) and 8.0 g/kg (Figure 2d and e), the ovaries of fish fed on the two plants had numerous vacuoles in the ooplasm and few pyknotic nuclei were observed in granulosa cells. Thecal layers were also thin characterized with increased thickness of connective tissue fibres.

4 | DISCUSSION

This study intended to assess the effects of *A. mossambicensis* and *A. indica* leaf powders on gonadal characteristics and histology of *O. niloticus*. Phytochemical screening of both plants revealed the presence of antifertility phytocompounds such as alkaloids and

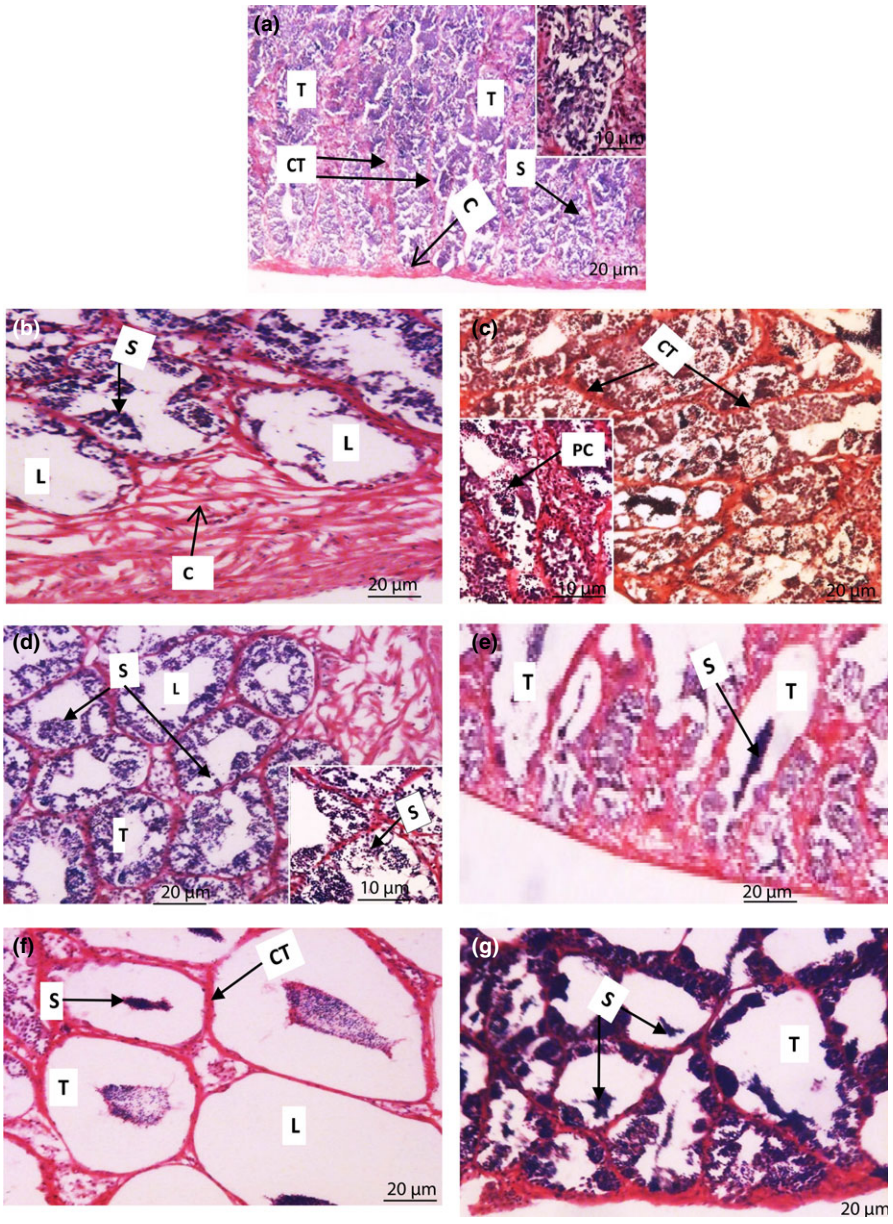


FIGURE 1 Representative cross-sections of testis tissue of *Oreochromis niloticus* showing changes after feeding experimental doses of *Aspilia mossambicensis* and *Azadirachta indica*. (a) Control seminiferous tubules (T) separated by connective tissue fibres (CT) testicular capsule (C), the lumen containing spermatozoa (S) at magnification of 20 and 40 for insert. (b) and (c) degenerating seminiferous tubules (T) separated by thick connective tissues (CT), testicular capsule (C). Degenerating cells exhibiting pallor cytoplasm (PC), and empty lumen (L) containing few aggregates of spermatozoa (S) from AM2 and AI2 fish, respectively, at magnifications of 20 for (b) and (c) and 40 for insert (c). (d) and (e) testis from AM4 and AI4 fish, respectively, showing testicular tissue atrophy, tufts of spermatozoa intermixed with inflammatory cells (S) in degenerating seminiferous tubules (T), at magnifications of 20 for (d) and (e) and 40 for insert (d). (f) and (g) testis from AM8 and AI8 fish, respectively, indicating severe testicular tissue atrophy, empty lumen (L) with very few clusters of spermatozoa (S), thickness of connective tissue (CT) separating the seminiferous tubules increased in the degenerating seminiferous tubules (T) at magnification of 20 [Colour figure can be viewed at wileyonlinelibrary.com]

flavonoids, which were more abundant in *A. indica* than in *A. mossambicensis*. Previous studies have shown that, *A. indica* is among the medicinal plants rich in phytocompounds such as alkaloids and flavonoids when compared to other medicinal plants (Harry-Asobara & Eno-Obong Okon, 2014; Kumar & Pandey, 2013; Obaineh, Aoludare, & Muhammad, 2013). The lower AF and RF of *O. niloticus* in the higher dose of *A. indica* is related to the presence of alkaloids and flavonoids in this plant. In the present study, *A. indica* had higher percentage of alkaloids and flavonoids. The presence of phytocompounds has been shown to alter the fertility of various organisms. For instance, lower fertility rate and fertility percentage were noticed in female mice treated with alkaloid ricinine from castor bean (*Ricinus communis*) seeds extract, which inhibited follicular development and ovulation (Al-Fartosi, AL-Haider, & AL-Maiyah, 2012). Likewise, total alkaloid fraction of *Aegle marmelos* decreased significantly the sperm concentration in male albino rats (Kumar, Rao, Madhusudhan, Reddy, & Prasad, 2011). Moreover, flavonoids (isoflavonoid formononetin) from certain species of clover-induced infertility syndrome in sheep (Middleton, Kandaswami, & Theoharis, 2000). Futhermore, flavonoids (genistein and daidzein) from soybean (*Glycine max*) altered sex ratio of *O. niloticus* by increasing the number of females due to their estrogenic effect (El-Sayed, Abdel-Aziz, & Abdel-Ghani, 2012). It is evident from these results that the presence of alkaloids and flavonoids were responsible for the observed antifertility and histological effects obtained in treated *O. niloticus*.

Dose-dependent decrease in AF for *O. niloticus* fed either on *A. mossambicensis* or *A. indica* concurs with the findings obtained by Jegede (2010) after exposing similar fish species to diets containing 1.0 to 4.0 g/kg of leaf powders. Similarly, Abdelhak et al. (2013) reported significantly lower AF after feeding *O. niloticus* with diets containing 60, 90 and 120 g/kg *Carica papaya* seed powders.

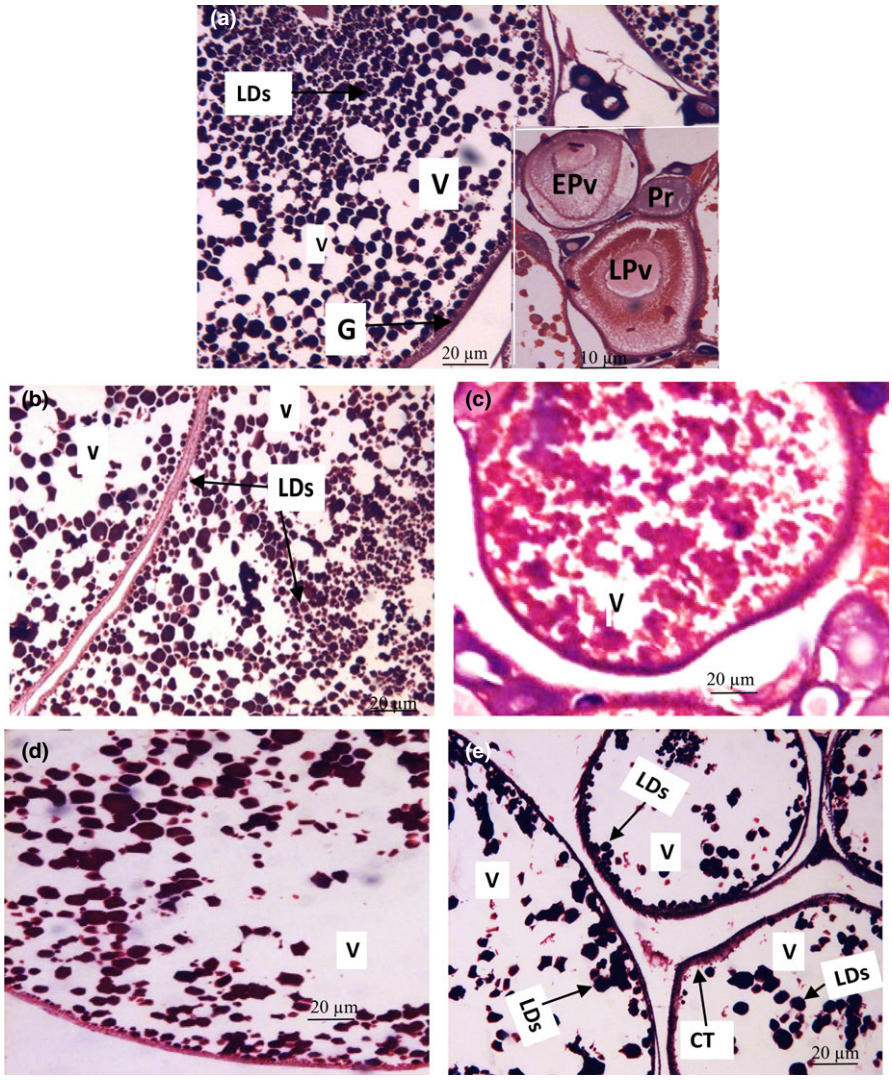


FIGURE 2 Representative cross-sections of ovary tissues from *Oreochromis niloticus* showing changes after feeding experimental doses of *Aspilia mossambicensis* and *Azadirachta indica*. (a) Ovary from control fish indicating ooplasm of vitellogenic follicle surrounded by a single layer of granulose cells (G), evenly distributed lipid droplets (LDs) and a few vacuoles (V). Insert: Primordial follicle (Pr); early previtellogenic follicle (EPv); late previtellogenic follicle (LPv) at magnifications of 20 and 40 insert. (b) and (c) ovaries from AM4 and AI4 fish indicating atretic follicles with few dispersed lipid droplets (LDs) and coalescence of vacuoles (V) in the ooplasm at magnifications of 20. (d) and (e) ovaries from AM8 and AI8 fish, respectively showing atretic follicles with thin thecal layers and increased thickness of connective tissue fibres (CT), lipid droplets (LDs) present only at the periphery and coalescence of vacuoles (V) in the ooplasm at magnification of 20 [Colour figure can be viewed at wileyonlinelibrary.com]

Gonadosomatic index values obtained from males fed either on *A. mossambicensis* or *A. indica* were lower (<2.4) compared to females (>3.7). Similar findings were reported by Jegede and Fagbenro (2008a) after feeding *Tilapia zilli* with diets containing 0.5 to 2.0 g/kg of *A. indica* leaf meal doses. In addition, *A. indica* aqueous leaf extract decreased significantly the weight of testes in male Albino rats (Sathiyara, Sivaraj, Vinoth Kumar, Devi, & Senthil Kumar, 2010). Dose-dependent decrease in AF of *O. niloticus* signifies that the two plants are capable of reducing gonadal characteristics of fish. We have reported recently that *A. mossambicensis* and *A. indica* leaf powders reduce offspring production in *O. niloticus* (Kapinga et al., 2018). Therefore, these findings suggest the possible use of the two plants in reducing gonadal characteristics indices of *O. niloticus*. In practice, *O. niloticus* farmers with access to the two plants can use them to reduce prolific breeding of fish.

The two plants induced histopathological changes in the gonads of treated *O. niloticus*. Dose-dependent degeneration of seminiferous tubules was observed from doses of 2.0 to 8.0 g/kg. The lumen of degenerating seminiferous tubules contained very few spermatozoa intermixed with inflammatory cells. In females, from doses of 4.0 to 8.0 g/kg of both *A. mossambicensis* and *A. indica*, numerous vacuoles were observed in the ooplasm of vitellogenic follicles, few pyknotic nuclei in granulosa cells whereas thecal layers were thin with increased thickness of connective tissue fibres. Similar findings were obtained by Obaroh and Nzeh (2014) and Jegede and Fagbenro (2008b) after feeding *O. niloticus* and *T. zillii* with diets supplemented with 0.5 to 8.0 g/kg and 0.5 to 2.0 g/kg of *A. indica*, respectively. The two plants appear to have more effects on males than females probably due to spermicidal effect of *A. indica* consistent with its use for contraceptive purposes in variety forms including pills, vaginal foam or creams (Bala, Arya, & Katare, 2014; Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002). These results imply that farmers interested in using the two plants to control prolific breeding should limit the inclusion dose to 2 g/kg to avoid severe effects on fish testis and ovaries.

In conclusion, this study has demonstrated that *A. mossambicensis* and *A. indica* leaf powders are capable of reducing gonadal characteristics indices and alter gonadal histology in *O. niloticus*. Fish fed on diets supplemented with *A. indica* demonstrated lower values of gonadal characteristics indices than those fed on *A. mossambicensis*.

Such variations could be attributed to higher phytochemical composition of *A. indica*. In practice, *O. niloticus* farmers interested in using the two plants to control prolific breeding should limit the dose inclusion to 2 g/kg to avoid severe effects on fish testis and ovaries. This study recommends further testing of *A. mossambicensis* and *A. indica* leaf powders on the newly hatched *O. niloticus* to investigate their effectiveness in inducing sex reversal.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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