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Assessment of the Inhibitory Effect of Selected Medicinal Plants Against *Aeromonas Sobria* in Nile Tilapia *(Oreochromis niloticus L.)*

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The study assessed the inhibitory effect of selected medicinal plants against *Aeromonas sobria* in Nile tilapia *Oreochromis niloticus*. *In vitro* experiment was used to examine the inhibitory effect of selected medicinal against *A. sobria.* In the *In vivo* experiment Nile tilapia was exposed at two concentrations of acacia, annatto and mango to evaluate the inhibitory effect against *Aeromonas sobria.* Results in *in vitro* experiment show that Zone of inhibition in acacia against the bacterium was only recorded in 100% extract concentration only. Extracts of annatto and mango in all concentration levels (25%-100%) showed zone of inhibition against the bacterium having the widest and narrowest diameter in 100% and 25% concentration, respectively. Using the highest bacterial suspension (10-7), extract of annatto and mango showed significant and wider zone of inhibition as compared to mango across extract concentrations.
In vivo experiment showed that disease symptoms such as red spots, haemorrhages and lesions have occurred in the challenged tilapia as early as Day 3. As compared to the initial TWBC, the study found out that there was apparent increase in TWBC count right after the appearance of the symptoms (three days after the challenge test).Significant increase in TWBC was only recorded in Treatment VII After 6 weeks prior to leaf extraction administration, there was decreased in the final TWBC of the experimental fish. Even not statistically significant, T5 (3% annatto) and T7 (3% mango) were effective in reducing the final TWBC count (T5 = reduced by 17.49 /mm3; T7 = reduced by 23.02/mm3) prior to infection. Four types of WBC were identified namely, monocytes, neutrophils, lymphocytes and basophil. Initial differential count revealed that percent composition of WBC was dominated by neutrophils, followed by lymphocytes and lastly monocytes

Keywords: Nile Tilapia, *Aeromonas sobria*, Inhibitory effect, White Blood Cells, Haemorrhages

Introduction

Aquaculture continues to be the fastest growing animal food-producing sector and to outpace population growth (FAO, 2005). Tilapia (*Oreochromis*

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niloticus) is one of many economical freshwater fishes that are cultured worldwide (Aboud, 2010). Herbivorous or omnivorous tilapia and carp species are cases in point, having been introduced throughout the tropics and accounting for about 80% of tropical inland aquaculture production (Arthur *et al*., 2010). It is well-known that water constitutes an important contamination route for microorganisms. This is especially true for *Aeromonas* spp. which are widespread in untreated and treated waters (Carvalho *et al*., 2012). *Aeromonas* spp. infections are probably the most common bacterial diseases diagnosed in cultured warm water fish (Majtan *et al*., 2012). The common occurrence of this disease relates to stress conditions such as poor water quality, overcrowding, or rough handling. Specifically, *A. sobria* in fishes causes hemorrhagic septicemia, fin rot, soft tissue rot and furunculosis (Aberoum and Jooyandeh, 2010). Other symptoms can include bleeding in the gills, the anal vent and the base of fins (Aberoum and Jooyandeh, 2010). Furthermore, they reported that epizootic ulcerative syndrome (EUS) caused by *A. sobria* resulted in great damage to fish farms in parts of southeast Asia such as in Bangladesh and India. *A sobria* was also the causative agent of fish disease in perch farm in Switzerland (Aberoum and Jooyandeh, 2010). According to Khan *et al*. (2008), bacteria under genus *Aeromonas* produce exotoxins to survive in undesirable condition which destroy the tissue and weaken the killing action of the host. Medicinal plants serve as powerful drugs nowadays due to their secondary metabolites constituents such as tannins, alkaloids and flavonoids (Ravikumar *et al*., 2012). These secondary metabolites have proven anti-bacterial potential. The antibacterial activity of mango for instance is due to its specific phytochemical components and the methanolic leaf extract of acacia showed a highly significant antibacterial activity against some bacteria (Stuart, 2011). Extract of annatto showed low Minimum Inhibitory Concentration (MIC) against *Escherichia coli* and a better MIC against *Bacillus cereus* (Stuart, 2011). The screening of active compounds from plants leads to discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Amara *et al*., 2008). Developing the potentials of these plant extracts against fish bacterial pathogens was a great help to the aquaculture industry (Ravikumar *et al*., 2012).

Objectives

The general objective of the study wasto evaluate the antibacterial potential of aqueous leaf extract of acacia, annatto and Indian mango against *A. sobria* infection in Nile tilapia. The specific objectives of the study were:

1) To determine the inhibitory effect of the plant extracts against *A. sobria* by measuring the zone of inhibition; and
2) To determine the effectiveness of the plant extracts in reducing the pathogenicity of *A. sobria* infection inNile tilapia.

Materials and methods

Cultivation of Bacteria

Pure culture of *A. sobria* was obtained from the Institute of Molecular Biology and Biotechnology of the University of the Philippines at Los Baños, Laguna. *A. sobria* was sub-cultured in Mueller Hinton Agar (MHA) plates and incubated at 37 ℃ for 18-24 hours. Stocks of bacterial culture in several test tubes of MHA were maintained for the succeeding studies.

Collection of Plant Samples

Matured and fresh leaves of acacia (*S. saman*), annatto (*B. orellana*) and Indian mango (*M. indica*) were collected within CLSU compound. Leaves of these medicinal plants were subjected to a hot water bath extraction.

Hot Water Bath Extraction

The collected leaves were washed thoroughly in running water. Twenty grams (20 g) of ground leaves were placed in a container with 100 mL of distilled water (1:6 dilution) (Angeles, 2004). The mouth of the container was sealed with aluminum foil. The container was placed in boiling water for an hour to start the extraction of the active ingredient. After cooling, the extract was filtered and placed in a container for immediate use in the experiment.

In Vitro Experiment of the Inhibitory Effect of Three Medicinal Plants against *A. Sobria* in Nile tilapia (*O. niloticus*)

Determination of Microbial Number

Serial dilutions of an 18-hour broth culture of *A. sobria* were prepared up to 10^{-7}. A volume of 0.1 mL (100 µl) was placed separately from 10^{-5}, 10^{-6} and 10^{-7} dilutions into the plates containing MHA. The inoculum was spread evenly on the surface of the agar using the inoculating loop. Each dilution was replicated thrice. The plates were incubated at 37 ℃ for 18-24 hours (Angeles, 2004). The average count of colonies in the three replicates per dilution was

taken as mean. The number of colony forming units (CFU) per mL of inoculum was computed by using the formula below:

For -5 dilution= mean x 10^{-5}
For -6 dilution= mean x 10^{-6}
For -7 dilution= mean x 10^{-7}

Preparation of the Extract Concentrations

Aqueous extract of the plants was diluted separately in sterile distilled water to obtain the concentrations of 0%, 25%, 50%, 75% and 100% (Angeles, 2004). Presented in Table 1 was the proportion of plant extract and distilled water.

Table 1. Proportion of plant extract and distilled water

Concentration (%)	Volume of plant extract (mL)	Volume of distilled water (mL)
0	0.00	5.00
25	1.25	3.75
50	2.50	2.50
75	3.75	1.25
100	5.00	0.00

Eradicant Test

Paper discs measuring 6 mm made from Whattman filter paper were prepared using paper puncher and were sterilized in an autoclave at 15 psi for 30 minutes. The sterilized discs were soaked into the various extract concentrations (Table 1) for 1 hour. *A. sobria* was grown in MHA plates. Using sterile forceps, prepared discs were impregnated on the surface of the inoculated plates in six replications. The plates were incubated at 37 ℃ for 18-24 hours in Complete Randomized Design. Inhibitory effects of the medicinal extracts against *A. sobria* were evaluated by measuring the diameter of the zones of inhibition express in mm using a ruler (Ruangpan and Tendencia, 2004).

Statistical Analysis

Differences in the zone of inhibition of the plant extracts at various concentrations were analyzed using ANOVA of the SPSS Software. For comparing sets of treatments, Tukey‟s test was used.

In Vivo Experiment Using the Three Medicinal Plants Against A. sobria Infection in Nile tilapia (O. niloticus)

Experimental Unit and Design

Circular plastic containers with volume capacity of 6 L were used in the experiment. The study used one-factor in RCBD with three replicates (Figure 1). The concentrations of the various treatments presented in Table 2 are adopted in the study of Angeles (2004). No aeration was provided in the experimental unit all throughout the course of the study.

T3	T6	T1	T4	T5	T2	T7
T7	T3	T2	T1	T5	T4	T6
T3	T6	T2	T7	T1	T4	T5

Figure 1. Experimental lay-out

Table 2. The various treatments that was used in the study with their corresponding description

Treatment	Description
1	No plant extract
2	1% Acacia extract
3	3% Acacia extract
4	1% Annatto extract
5	3% Annatto extract
6	1% Mango extract
7	3% Mango extract

Note: 1% = Mix 0.5 L medicinal leaf extract plus 49.5 L water in the aquarium
3% = Mix 1.5 L medicinal leaf extract plus 48.5 L water in the aquarium

Challenge Test

After one week of acclimatization, the experimental fish was challenged with 0.2 mL of 10^{-6} CFU per mL of *A. sobria* through intraperitoneal injection (Angeles, 2004). Manifestations of *A. sobria* infection in the fish in each experimental unit were observed daily after the challenge test. The challenge test lasted for 6 weeks. During this period, the experimental fish was continually fed with commercial feeds

Leaf Extract Administration

The different leaf extract concentrations (Table 2) were administered only when the fish showed clinical manifestations of the bacterial infection.

Collection of Blood and Analysis

Fish blood samples were collected before and after the challenge test, and before the administration of the leaf extract. Approximately, 200 µl of blood sample was collected from each fish per experimental unit by puncturing blood vessels at the gill region using a 1 mL disposable syringe with gauge # 25 needle (Angeles, 2004). The collected blood was used for WBC and differential WBC counts.

WBC count

A 100 µl blood was added with 2 mL of Turk‟s solution previously placed in a petri dish. The mixture was mixed gently before a volume of 2 µl was put in a hemocytometer counting chamber. A drop of blood was spread thinly on the slide. The smear was air dried by waving it rapidly. The slide was stained using Wright Stain (Angeles, 2004).

The total WBC count per mm^3 of the hemocytometer was calculated by using the formula:

$$\text{Total WBC per mm}^3 = \frac{\text{WBC's counted x Dilution (1:20)}}{\text{Volume 0.4}}$$

Differential WBC count

Differential WBC count was done using the oil immersion objective of the photomicroscope. Each individual cell type (i.e. lymphocytes, monocytes, eosinophil, basophil and neutrophils) was recorded on a tally sheet until a total of 100 cells were counted. The blood cells were differentiated based on the cell type. The number of cells counted was expressed in percentage (Angeles, 2004).

Water Quality Parameters and Analysis

Water quality parameters such as temperature, pH and dissolved oxygen (DO) were analyzed daily from 9-10 am and 2-3 pm using YSI multi-parameter equipment. Total ammonia nitrogen was analyzed on a weekly basis.

Statistical Analysis

Data on WBC and differential WBC counts were subjected to square root and arcsin transformations, respectively. The transformed data were analyzed using the Analysis of variance to determine significant differences within treatments. Tukey‟s test was used for the comparison of treatment means. Meanwhile, T-test was used in comparing the means before and after the challenge test. The data were analyzed using the SPSS Software.

Results

In Vitro Experiment of the Inhibitory Effect of Acacia, Annatto and Mango Extract against Aeromonas sobria in Nile tilapia (Oreochromis niloticus)

Zone of Inhibition

The potential of three medicinal plants (Acacia, *Samanea saman*; Annatto, *Bixa orellana*; Mango, *Mangifera indica*) in four extract concentrations (25, 50, 75 and 100%) against *Aeromonas sobria* (3.25×10^{2}, 10^{-5}; 9×10^{2}, 10^{-6} and 1.4×10^{3}, 10^{-7}CFU/mL)was tested through measuring the zone of inhibition. Presented in Figure 2 was the observed zone of inhibition of the plant extracts against the bacterial suspension. Zone of inhibition in acacia against the bacterium was only recorded in100% extract concentration. Extracts of annatto and mango in all concentration levels (25-100%) showed zone of inhibition against the bacterium having the widest and narrowest diameter in 100% and 25% concentration, respectively. At $9x10^{2}$ (10^{-6}) and 1.4×10^{3} (10^{-5}) CFU/mL bacterial suspension, the zone of inhibition of annatto and mango was significantly wider as compared to acacia across percentage of extract tested. Using the highest bacterial suspension (3.25×10^{2}, 10^{-7}) CFU/mL), extract of annatto and mango showed significant and wider zone of inhibition as compared to mango across extract concentrations. Meanwhile, the zone of inhibition of annatto was significantly higher to mango except on 25% concentration.

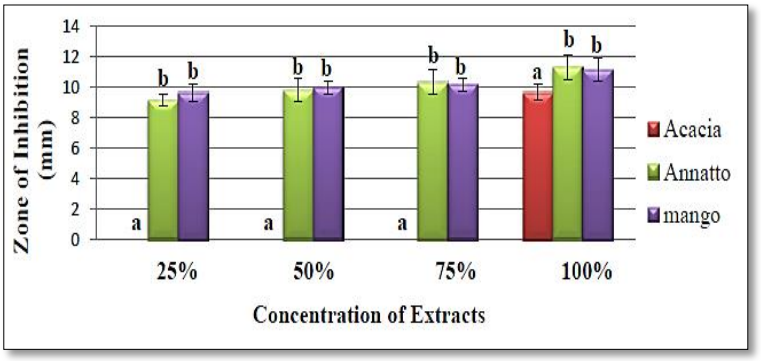

Figure 2a.Inhibitory zone of the three plant extracts against 10^{-5}CFU/mL *Aeromonas sobria* (Different letter was significant at $p<0.05$).

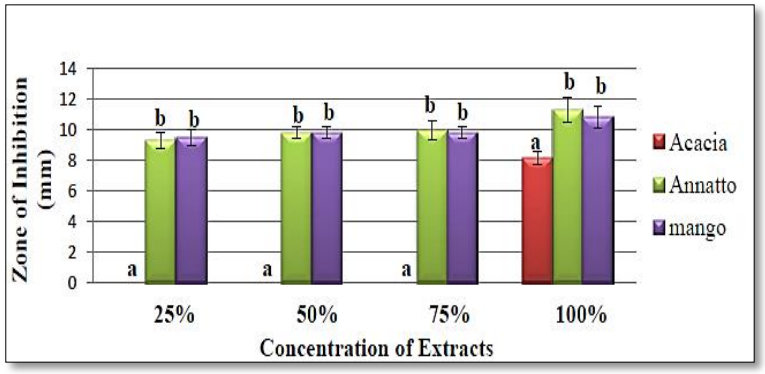

Figure 2b.Inhibitory zone of the three plant extracts against 10^{-6}CFU/mL *Aeromonas sobria* (Different letter was significant at $p<0.05$).

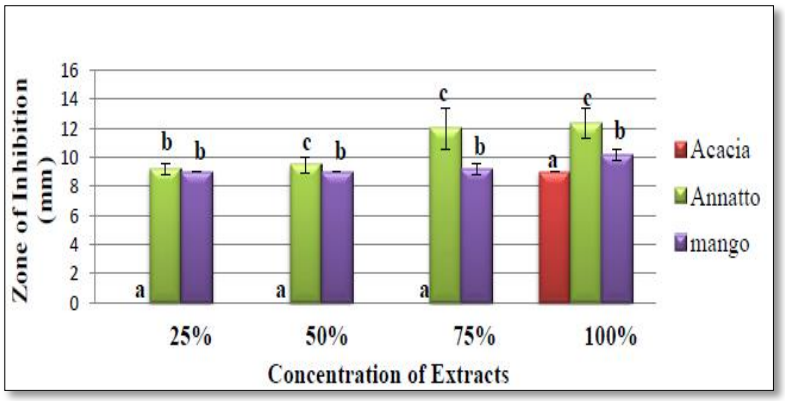

Figure 2c.Inhibitory zone of the three plant extracts against 10^{-7}CFU/mL *Aeromonas sobria* (Different letter was significant at $p<0.05$).

In Vivo Experiment Using Acacia, Annatto and Mango Extract Against Aeromonas sobria Infection in Nile tilapia (Oreochromis niloticus)

General Condition of the Experimental Fish

After three days of challenge test, majority of the experimental fishes had red spots at the head region accompanied by the reddish color of the caudal fin. Disease manifestations such as fin erosion (particularly caudal fin), hemorrhages and lesions (around operculum) were observed (Figure 3).

These manifestations were observed mostly in the experimental fish. Fish mortality was recorded as early as 5th day of the experiment; the second occurrence of mortality was in 12th day.

Representative fish for each treatment at the termination of the experiment was presented in Figure 4.

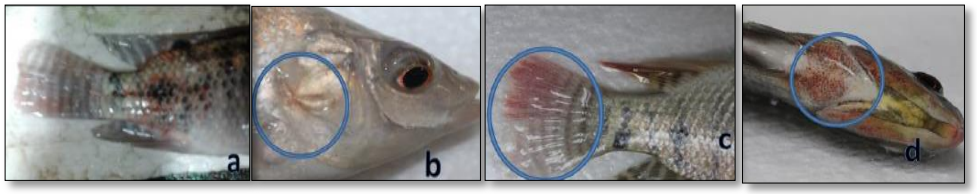

Figure 3. Symptoms of disease manifested in *O. niloticus* infected by *A. sobria* (a) body hemorrhages; (b) lesions at the operculum; (c) reddening of caudal fin and fin erosion; (d) red spots at the head region.

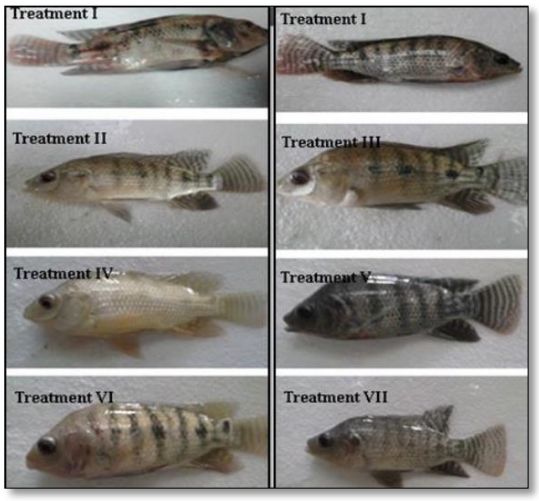

Figure 4. Representative fish for each treatment at the termination of the experiment.

Total White Blood Cell Count

Presented in Table 3 was the linearized initial total white blood cell (TWBC) count in Nile tilapia across treatments. Statistical analysis showed that

TWBC count was comparable among treatments. Result has indicated that there was no variation in the initial TWBC of the experimental fish.

Table 3.Initial TWBC count in Nile tilapia.

Treatment	Initial TWBC
I (control; no plant extract)	186.35±23.84a
II (acacia; 1% extract)	199.09±17.80a
III (acacia; 3% extract)	179.45±16.98a
IV (annatto; 1% extract)	182.43±46.39a
V (annatto; 3% extract)	192.09±9.60a
VI (mango;1% extract)	206.74±8.37a
VII (mango; 3% extract)	193.07±10.88a

Note: Different letter was significant at $p<0.05$

As stated in the previous page, symptoms of disease have appeared three days after the challenge test. Blood from the experimental fish in each treatment was pulled for TWBC counting. Result of the count was presented in Table 4.

Table 4.TWBC count in Nile tilapia right after disease manifestations.

Treatment	TWBC
I (control; no plant extract)	192.12±18.04a
II (acacia; 1% extract)	212.15±42.99a
III (acacia; 3% extract)	192.41±12.40a
IV (annatto; 1% extract)	207.74±46.07a
V (annatto; 3% extract)	204.56±2.75a
VI (mango;1% extract)	206.00±16.31a
VII (mango; 3% extract)	202.52±12.41a

Note: Different letter was significant at $p<0.05$

Comparing the result of Table 3 and Table 4, there was apparent increase in TWBC count (except Treatment VI) three days after the challenge test. Statistical comparison showed that increase in TWBC in most treatments was not significant; significant increase inTWBC was only recorded in Treatment VII (193.07±10.88vs 202.52±12.41). The diseased fish was treated using 1% and 3% leaf extract of acacia, annatto and mango. After six weeks, final counting of TWBC was done and the result was provided in Table 5.

Table 5.Final TWBC count in Nile tilapia

Treatment	Final TWBC
I (control; no plant extract)	193.57±22.12a
II. (acacia; 1% extract)	202.40±8.10a
III (acacia; 3% extract)	196.32±14.42a
IV (annatto; 1% extract)	196.11±11.15a
V (annatto; 3% extract)	187.07±20.71a
VI (mango;1% extract)	190.65±29.21a
VII (mango; 3% extract)	178.21±23.16a

Note: Different letter was significant at $p<0.05$

After 6 weeks prior to leaf extraction administration, there was decreased in the final TWBC of the experimental fish (Table 5). This only implied that there was a possibilityof reduced infection as brought by the inoculated bacterium. In T1 (control), the final TWBC (Table 5) was reduced only by less than a unit (0.65/mm3TWBC) as compared to the TWBC before (Table 4) the extract was administered. Treatments with 3% extract administration were better than 1% extract concentration based upon reduction in the final TWBC (Table 4 and 5). Even not statistically significant, T5 (3% annatto) and T7 (3% mango) were effective in reducing the final TWBC count (T5 = reduced by 17.49/mm3; T7 = reduced by 23.02/mm3) prior to infection (Table 4 and 5).

Differential Count

Hematological composition

Differential count was done to determine the percent composition of the WBC in the experimental fishes. Fourtypes of WBC were identified namely, monocytes, neutrophils, lymphocytes and basophil (Figure 5). The latter was very rare and only existed in two treatments, thus, it was not included in the discussion.

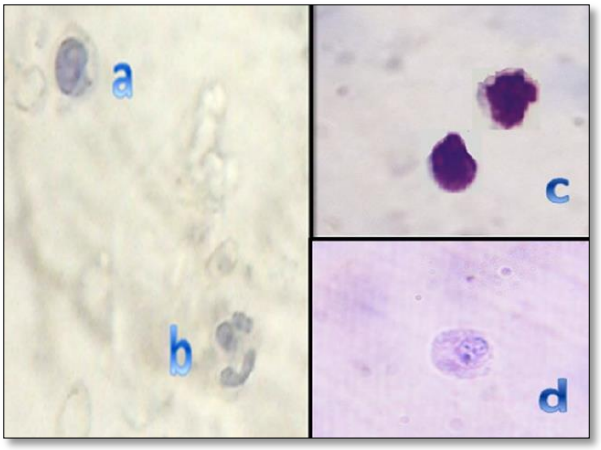

Figure 5.Different types of white blood cell (a) monocyte, (b) neutrophils (c) lymphocyte and (d) basophil

Initial differential count revealed that percent composition of WBC was dominated by neutrophils (48.00-51.67%), followed by lymphocytes (37.40-46.83%) and lastly monocytes (8.17-10.67%) (Table 6). Percent monocytes were significantly lower as compared to the two types of WBC. Compared to neutrophils (65%) and lymphocytes (20-25%).

Table 6.Comparison of percent composition of WBC during initial count.

Treatment	Monocyte (%)	Lymphocyte (%)	Neutrophils (%)
I (control; no plant extract)	10.67±4.55a	38.83±2.93b	50.50±5.68c
II (acacia; 1% extract)	7.50±4.09a	44.50±5.92b	48.00±6.29b
III (acacia; 3% extract)	9.83±5.00a	38.50±6.72b	51.67±3.98c
IV (annatto; 1% extract)	9.33±2.07a	43.50±3.94b	50.33±7.74b
V (annatto; 3% extract)	8.17±3.82a	41.50±2.74b	50.33±5.65c
VI (mango;1% extract)	13.17±2.79a	37.33±3.72b	49.50±3.67c
VII (mango; 3% extract)	9.67±3.44a	39.83±5.71b	50.50±7.50c

Note: Different letter was significant at $p<0.05$

Three days after the challenge test, there was remarkable changes in the WBC profile of the infected fish (Table 7). Significant increase in percent monocytes after the appearance of symptoms was recorded in T2 (7.50±4.09 vs 17.50±5.61), T4 (9.33±2.07 vs 17.33±4.23), T5 (7.60±3.97 vs 15.40±4.83) and T7 (9.67±3.44 vs 15.67±2.50). Percent neutrophils also increase but statistically not significant. For lymphocytes, all treatments showed significant decrease right after the appearance of symptoms of diseases ($p<0.05$) (Figure 6).

Table 7. Comparison of percent composition of WBC right after manifestation of disease.

Treatment	Monocyte (%)	Lymphocyte (%)	Neutrophils (%)
I (control; no plant extract)	15.00±4.60a	24.00±7.72a	61.00±10.35b
II (acacia; 1% extract)	17.50±5.61a	27.03±4.88b	54.67±6.98c
III (acacia; 3% extract)	14.17±2.48a	31.17±5.27b	54.67±6.22c
IV (annatto; 1% extract)	17.33±4.23b	29.83±4.79c	52.67±5.13d
V (annatto; 3% extract)	15.40±4.83a	30.60±4.28b	54.00±7.84c
VI (mango;1% extract)	15.17±3.06a	31.00±3.27b	53.33±5.05c
VII (mango; 3% extract)	15.67±2.50a	32.83±6.08b	51.67±5.20c

Note: Different letter was significant at $p<0.05$

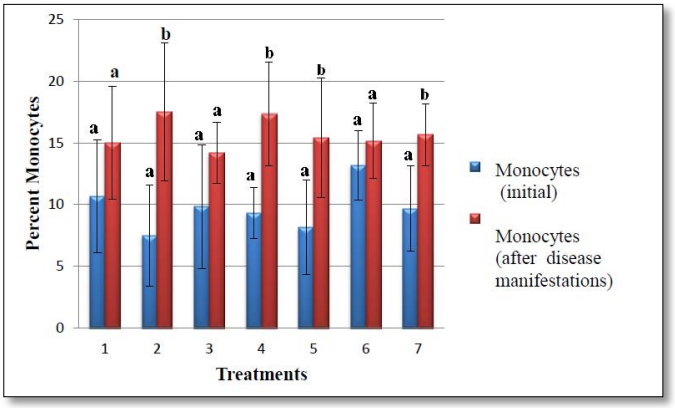

Figure 6a. Comparison of percent monocytes during initial count and after the manifestation of disease in Nile tilapia (Different letter was significant at $p<0.05$).

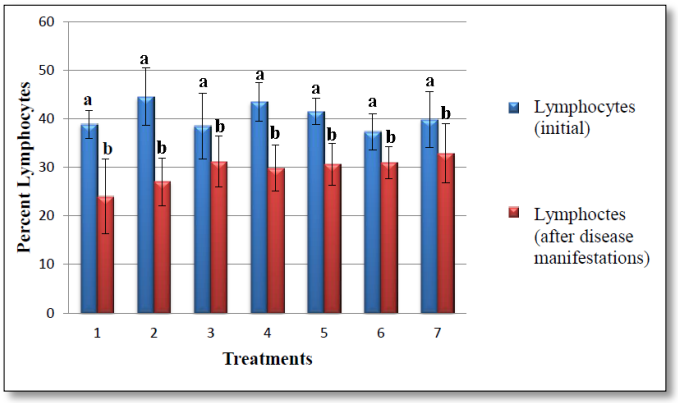

Figure 6b.Comparison of percent lymphocytes during initial count and after the manifestation of disease in Nile tilapia (Different letter was significant at $p<0.05$).

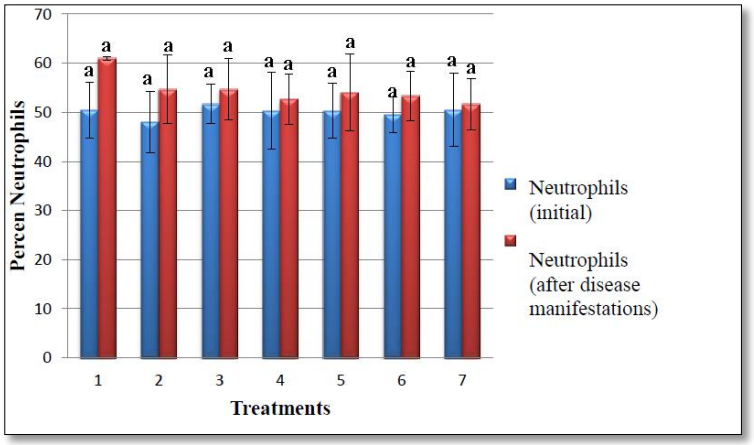

Figure 6c. Comparison of percent neutrophils during initial count and after the manifestation of disease in Nile tilapia(Different letter was significant at $p<0.05$)

There was notable increase in percent lymphocytes six weeks after the administration of the plant extract (Table 8). Significant increase was recorded in all treatments that exhibited continued appearance of the symptoms. Almost 40% increase in final lymphocyte was recorded in T1 (control; 25.00±7.44 vs 41.40±1.67). Symptoms in fish treated with 3% acacia (T3) and 3% mango (T7) have stopped appearing four weeks after the application of the extract. This might be the reason why there was small increase only in final lymphocyte count (Figure 7).

Meanwhile final monocyte counts across treatments were lower as compared to the previous count done right after the manifestation of disease. There was also decrease in percent neutrophils, but significant decrease was recorded in T1, T3, T4 and T6 (Figure 7).

Table 8.Comparison of percent composition of WBC after the administration of plant extracts.

Treatment	Monocyte (%)	Lymphocyte (%)	Neutrophils (%)
I (control; no plant extract)	21.00±8.51b	41.40±1.67c	37.40±9.53c
II (acacia; 1% extract)	15.60±3.05a	41.00±4.95b	43.40±3.65b
III (acacia; 3% extract)	16.83±7.22a	36.33±8.82b	46.83±7.19b
IV (annatto; 1% extract)	20.40±3.13a	40.60±3.65b	39.00±5.34b
V (annatto; 3% extract)	15.40±3.36a	41.80±5.63b	42.80±6.87b
VI (mango;1% extract)	20.40±3.21a	40.00±6.04b	39.60±5.81b
VII (mango; 3% extract)	12.00±5.16a	41.75±7.63b	46.25±6.99b

Note: Different letter was significant at $p<0.05$

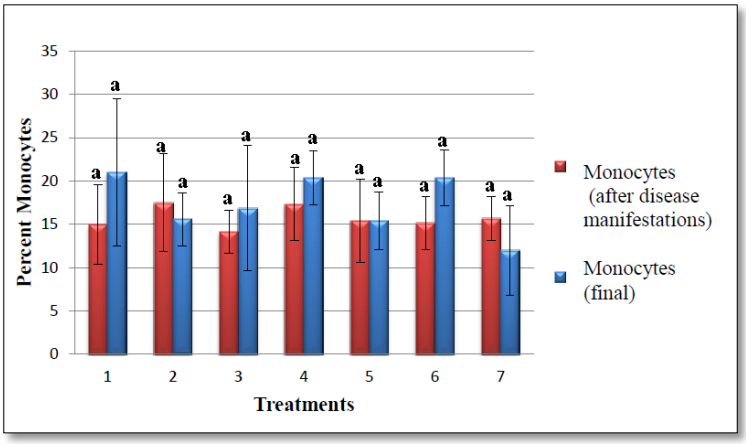

Figure 7a.Comparison of percent monocytes after the manifestationof disease and final count in Nile tilapia (Different letter was significant at $p<0.05$).

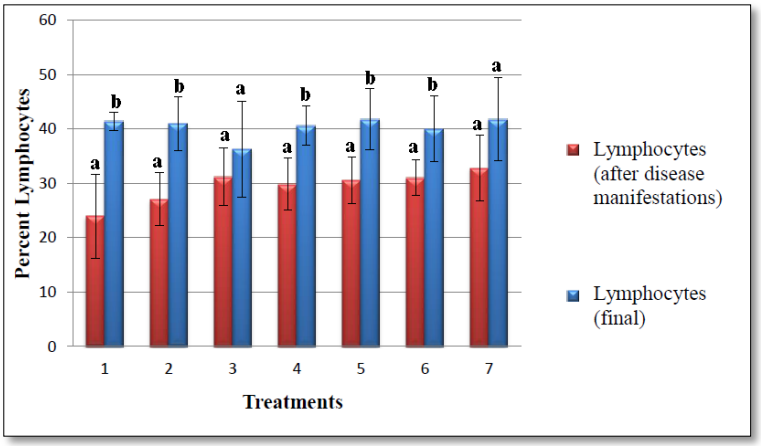

Figure 7b.Comparison of percent lymphocytes after the manifestationof disease and final count in Nile tilapia (Different letter was significant at $p<0.05$).

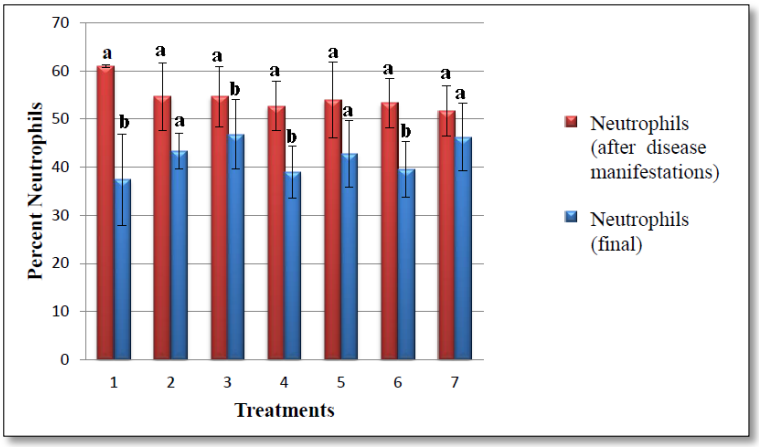

Figure 7c.Comparison of percent neutrophils after the manifestationof disease and final count in Nile tilapia (Different letter was significant at $p<0.05$).

Water Quality

Four water quality parameters were measured that includes temperature, pH, dissolved oxygen (DO) and total ammonia nitrogen (TAN). Daily and weekly readings of temperature, pH, DO and TAN were done.

Average weekly readings of temperature were below optimum, as low as 21℃ in the morning and as high as 27℃ in the afternoon. Lowest temperature readings were recorded in Week 6 of the experiment. Majority of DO readings were optimum, with lowest reading of 3 mg/L in some treatments during Week 3 and Week 4 only. Readings of pH were optimum from Week 1 until Week 6, with lowest and highest readings of 7.8 and 8.7. Lastly, TAN readings were optimum except for Week 1 having 6.3 mg/L as the highest

Discussion

The study was composed of two parts: (1) *In vitro* experiment of the inhibitory effect of acacia, annatto and mango extract against *Aeromonas sobria* in Nile tilapia (*Oreochromis niloticus*) and (2) *In vivo* experiment using the above plant extracts against the bacterium infection in Nile tilapia. In the first experiment, the potential of the three plant extracts was tested by measuring the zone of inhibition. For the second experiment, the response of WBC was determined in tilapia challenged with the bacterium and afterwards treated with various concentrations of the plant extract.

Zone of inhibition in acacia against the bacterium was only recorded in100% extract concentration.Extracts of annatto and mango in all concentration levels (25-100%) showed zone of inhibition against the bacterium having the widest and narrowest diameter in 100% and 25% concentration, respectively. Using the highest bacterial suspension (10-7), extract of annatto and mango showed significant and wider zone of inhibition as compared to mango across extract concentrations.

For experiment 2, disease symptoms such as red spots, hemorrhages and lesions have occurred in the challenged tilapia as early as Day 3.Cipriano (2001) observed that 72.02% of *A. hyrophilla* isolates and 63 % of *A. sobria* were virulent to the fish by intramuscular challenge, bul all strains of *A. caviae* were not virulent.

Pathological conditions attributed to members of the motile aeromonad complex may include dermal ulceration, tail or fin rot, ocular ulcerations, erythrodermatitis, hemorrhagic septicemia, red sore disease, red rot disease, and scale protrusion disease (Cipriano, 2001). He also stated that chronic motile aeromonad infections manifest themselves primarily as ulcerous forms of disease, in which dermal lesions with focal hemorrhage and inflammation are apparent (Cipriano, 2001). The gross lesions of the skin varied and depigmented erosions of various sizes, occurring mainly on the sides near the gill covers (Rehulka, 2002). As compared to the initial TWBC, the study found out that there was apparent increase in TWBC count right after the appearance of the symptoms (three days after the challenge test). The same result was noted by Elkamel and Mosaad (2012) who worked in Nile tilapia challenged with *A. hydrophila*. Significant increase in TWBC was only recorded in Treatment VII (193.07 ±10.88vs 202.52 ±12.41). Haniffa and Abdul (2010), also observed an increase in the number of white blood cell count after *Channa striatus* was injected with *Aeromonas hydrophila*, enhancing the defense mechanism of the fish. After 6 weeks prior to leaf extraction administration, there was decreased in the final TWBC of the experimental fish. In T1 (control), the final TWBC was reducedonly by less than a unit (0.65/mm^3TWBC) as compared to the TWBC before the extract was administered.

Even not statistically significant, T5 (3% annatto) and T7 (3% mango) were effective in reducing the final TWBC count (T5 = reduced by 17.49/mm^3; T7 = reduced by 23.02 /mm^3) prior to infection.

Four types of WBC were identified namely, monocytes, neutrophils, lymphocytes and basophil. Initial differential count revealed that percent composition of WBC was dominated by neutrophils, followed by lymphocytes and lastly monocytes (8.17-10.67%).Three days after the challenge test, there was significant increase in percent monocytes and lymphocytes; percent

neutrophils also increase but statistically not significant, according to Al-Zubaydi, (undated), monocytes are the largest of the WBCs but make up only of 3-8% of the total WBCs in the blood. The differential leucocytic-count is an indicator of heath in fish. Neutrophils are said to be the first line of defense of fish. According to Cuesta *et al.* (2005) as cited by Alishahi and Jangeran (2012), water and ethanolic-extracts of propolis (honey bee product) increased the percentage of neutrophils in gilthead sea bream.

Significant increase in percent lymphocyte 6weeks after the administration of the plant extract was observed in most of the treatment, especially T1 (control). The lymphocytes are the most dominant leucocyte type in the blood of *Clarias albopunctatus* and decrease in the neutrophils, monocytes and eosinophil counts was observed, these observations are indications of the mobilization of the body‟s defense system (Oluah and Mgbenka, 2004). According to Abd-El-Rhman *et al.* (2009) as cited by Alishahi and Jangeran (2012), they reported a decrease in the neutrophils under oral administration of propolis (honey bee product) in tilapia.

Symptoms in fish treated with 3% acacia (T3) and3% mango (T7) have stopped appearing 4weeks after the application of the extract. This might be the reason why there was small increase only in final lymphocyte count. Meanwhile final monocyte and neutrophils counts across treatments were lower as compared to the previous count maderight after the manifestation of disease.

The study concluded the following: 1) Extracts of mango and annatto had potential against *A. sobria* based upon the observed zone of inhibition; 2) Symptoms such as red spots, hemorrhage and lesions, and fin rot were common in *A. sobria*infected tilapia; mortality was recorded as early as 5days before challenge test; 3) There was reduction in the TWBC of infected tilapia once treated with plant extracts, and higher dose (3%) was found more effective; 4) There was reduction in percent lymphocytes onthe fish treated with 3%acacia and 3% annatto after 6weeks experiment; and 5) The three plant extracts had potential *in vitro* and *in vivo*but 3% annatto was more recommended to use against the bacterium.

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