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Evaluation of *Moringa oleifera, Psidium guajava* Leaves Extracts and Oxytetracycline on Tissues Bacteriological Changes of *Clarias gariepinus* Juveniles

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Abstract
The present study evaluated the effect of drumstick (*Moringa oleifera*) leaves and guava *(Psidium guajava)* leaves extracts and oxytetracycline on tissue bacteriological characteristics of *Clarias gariepinus* juveniles. Fish (8.90 ± 0.01g) were assigned to nine treatments with two replicates each. The treatment groups had different levels of *M. oleifera* and *P. guajava* leaves (1%, 2% and 3%) respectively and oxytetracycline (15 and 30mg/kg diet), the control group was without drumstick and guava leaves and oxytetracycline. Diet contained 40% crude protein, fed to the fish at 3% body weight twice daily for 56 days. Microbiological analyses of water and fish tissue (skin, gill, intestine and liver) were investigated using standard methods. Data were analyzed using ANOVA at $p<0.05$. The results of the study showed that all *M. oleifera*, *P. guajava* and oxytetracycline levels had decreased enterobacteriacea and total viable counts in water and fish tissues (skin, gill, intestine and liver) as inclusion rate increased and as months increased when compared to the control at 0, 4 and 8 weeks. It can be suggested that dietary supplementation of *M. oleifera*, *P. guajava* leaves extracts and oxytetracycline to fish diet can reduce bacterial growth and improve fish health.

Keywords: *Moringa oleifera*, *Psidium guajava*, *Clarias gariepinus*, Oxytetracycline, Bacteria

Introduction

Due to increase in the intensification and commercialization of aquaculture production, infectious diseases are a major problem causing heavy loss to the fish farming industry (Bondad- Reantaso *et al*., 2005). Intensive culture and adverse environmental conditions are often attributed to disease due to immune suppression or physiological stress (Cabello, 2006). The impact of intensive use of antimicrobial agent worldwide for prophylactic and therapeutics purposed has been associated with the increase of bacterial resistance in the exposed microbial environment (Sanjoy *et al.*, 2012).

Considering the potential threat of disease on human and animal health, issues associated with the use of antibiotics in disease management should therefore focus on environmental – friendly preventative methods such as the use of natural medicinal plants. Drumstick and guava leaves as natural medicinal plants could be considered as phytobiotics in culture fish as they possess high antimicrobial effects but there is a dearth of information on their utilization in fish as well as the mechanism of action.

The aim of the current study was to evaluate the *in vivo* potential effect of *M. oleifera* and *P. guajava* leaves on performance and antimicrobial activity for *C. gariepinus*.

Materials and Methods

Plant Preparation and Extraction

The *P. guajava* and *M. oleifera* leaves were used in the study and the extraction was carried out using the method of Ajaiyeoba and Fadare (2006). The air-dried *P. guajava* and *M. oleifera* leaves were ground with a hammer mill and 200g of fine powder of *P. guajava* and *M. oleifera* were soaked in 1000ml of 80% methanol and1000ml of 95% ethanol for 48 hours respectively. These plants were properly mixed with ethanol and methanol at constant interval, filtered using sterile muslin cloth after which the extract was obtained, air-dried and stored at 25^0C until required.

Experimental Procedures and Feeding Trials

Each treatment has two replicates, 20 fish per replicate with mean initial body weight of 8.90 ± 0.01g and uniform-sized fish were selected from 460 juveniles. Weighed and distributed in experimental bowl. The fish were acclimated for fourteen (14) days in the experimental bowl before the experiment. The experiment lasted for Eight (8) weeks during which the fish was fed at 3% body weight daily. The diet per day was divided into two: 1.5% given in the morning between 8.00 - 9.00 and 1.5% in the evening by 5pm. Measurement of the weight changes was performed fortnightly and the feeding rate adjusted fortnightly according to the new body weight.

Preparation of Experimental Diets

The mean proximate composition of the experimental diet was 40.08% crude protein, 6.15% ether extract, 10.31% ash, 8.22% moisture, and 35.24% NFE. Nine experimental diets were prepared by incorporating different levels of *M. oleifera* and *P. guajava* leaves (1%, 2% and 3%) respectively and oxytetracycline (15 and 30mg/kg diet), the control group was without drumstick and guava leaves and oxytetracycline. Feed ingredients such as fishmeal, soybean, maize, starch, vegetable oil, Di calcium phosphate (DCP), and vitamin- mineral premix were added and the dry ingredients mixed thoroughly in a mixer. Water was added and the resulting dough pelletted using ¼ mm die mincer of Hobalt A-200T pelleting machine. The pellets were sun – dried, and stored in airtight containers at room temperature to prevent mould formation until required.

Microbiological Analysis

Water samples from the aquaria and fish samples (skin, liver, gill and intestine) were collected monthly (4 and 8 weeks) in sterile glass bottles. Peptone water 0.1% was used for serial dilution. 1ml of water sample and 1g of fish sample was added to 9ml sterile peptone water separately to 10-1 and then diluted to 10-4. Each diluent (1ml, 1g) was poured in two petri dishes; one received plate count agar (MacConkey) for enterobacteriacea counts using the pure plate count method according to the standard methods for the examination of water and waste water (APHA, 1985), the second Petri dish received Nutrient agar for total viable counts for water sample and fish sample respectively according to Hitchins *et al.* (1995). Petri dishes were gently tapped on the sides for a few times. Petri dish for enterobacteriacea counts and total viable counts were incubated at 37^{0}C for 24hr. After incubation of water and fish sample dishes the colonies were counted using colony counter. Total viable counts and enterobacteriacea counts were determined, the result were expressed in $\log_{10}$ CFU/ml and $\log_{10}$ CFU/g for water and fish respectively.

Statistical Analysis

Microbial loads of water, skin, liver, gill and intestine resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences version 20). Duncan New Multiple range test was used to compare differences among individual mean at $P < 0.05$.

Results

Microbial Analysis of Water

The values of enterobacteriacea and total viable counts observed at 4 week were higher than the value observed at 8 week. The microbial loads in the treatments decreased as the months increased and they were no significant difference ($p>0.05$) among the treatments (see Table 1).

Table 1: Microbial loads ($\log_{10}$CFU/ml) of water samples treated with drumstick leaves, guava leaves and oxytetracycline.

Treatments	4 weeks		8 weeks	
	Enterobacteriacea counts	Total viable counts	Enterobacteriacea counts	Total viable counts
CTRL	6.66±0.15^a	6.89±0.11^a	6.85±0.15^a	7.33±0.35^a
DL2 (1%)	6.39±0.11^a	6.41±0.06^a	6.30±0.00^a	6.80±0.15^a
DL3 (2%)	5.58±0.02^a	6.49±0.20^a	5.75±0.13^a	6.24±0.06^a
DL4 (3%)	6.30±0.13^a	6.51±0.00^a	5.97±0.39^a	6.29±0.21^a
GL5 (1%)	6.06±0.01^a	6.61±0.00^a	6.04±0.04^a	6.98±0.11^a
GL6 (2%)	6.06±0.02^a	6.62±0.00^a	6.09±0.09^a	6.29±0.11^a
GL7 (3%)	6.06±0.35^a	6.59±0.00^a	6.00±0.00^a	6.89±0.06^a
OXY8 (15mg/kg diet)	6.45±0.15^a	7.18±0.45^a	6.24±0.06^a	7.35±0.15^a
OXY9 (30mg/kg diet)	6.39±0.06^a	6.54±0.09^a	6.04±0.04^a	6.29±0.11^a

Mean followed by the same letter in the column were not significantly different ($p > 0.05$), DL = Drumstick Leaves, GL = Guava Leaves, OXY = Oxytetracycline

Microbial Analysis of Fish Tissue

The microbial loads of skin, liver, gill and intestine of the control was significantly higher ($P<0.05$) than the treated groups at 4 and 8 weeks. The values of enterobacteriacea counts and the total viable counts decreased as the inclusion level increased as the months increased (see Table 2).

Table 2: Microbial loads (log_{10}CFU/g) of fish tissue treated with drumstick leaves and guava leaves

Treatment	Fish sites	4 weeks		8 weeks	
		Enterobacteriacea counts	Total Viable Counts	Enterobacteriacea counts	Total Viable Counts
CTRL	Skin	6.43±0.02^{g}	6.63±0.04^{e}	6.25±0.10^{f}	6.48±0.01^{f}
	Liver	5.82±0.00^{f}	5.94±0.00^{g}	5.62±0.02^{g}	5.66±0.04^{e}
	Gill	6.32±0.01^{f}	6.44±0.03^{e}	6.15±0.12^{f}	6.22±0.03^{g}
	Intestine	6.11±0.03^{e}	6.20±0.01^{h}	6.01±0.06^{f}	6.08±0.16^{f}
DL 2	Skin	6.21±0.04$_{d}$	6.34±0.10^{c}	6.03±0.03^{d}	6.11±0.03^{c}
	Liver	5.53±0.02^{c}	5.62±0.02^{cd}	5.15±0.03^{cd}	5.41±0.01^{bc}
	Gill	6.10±0.25^{d}	6.21±0.03^{c}	5.74±0.04^{b}	5.99±0.04^{d}
	Intestine	5.96±0.10^{cd}	6.02±0.15^{ef}	5.79±0.06^{d}	5.89±0.08^{d}
DL 3	Skin	6.01±0.01^{b}	6.21±0.03^{b}	5.86±0.02^{c}	5.96±0.04^{b}
	Liver	5.37±0.00^{b}	5.58±0.10^{bc}	5.11±0.01^{bc}	5.38±0.05^{b}
	Gill	5.97±0.03^{c}	6.05±0.25^{b}	5.69±0.02^{b}	5.79±0.07^{b}
	Intestine	5.73±0.20^{abc}	5.84±0.30^{c}	5.49±0.06^{a}	5.62±0.00^{a}
DL 4	Skin	5.85±0.00^{a}	5.98±0.01^{a}	5.61±0.02^{a}	5.71±0.01^{a}
	Liver	5.17±0.10^{a}	5.31±0.02^{a}	4.97±0.04^{a}	5.08±0.04^{a}
	Gill	5.78±0.02^{a}	5.89±0.04^{a}	5.61±0.05^{a}	5.63±0.03^{a}
	Intestine	5.59±0.04^{a}	5.68±0.12^{a}	5.48±0.12^{a}	5.59±0.02^{a}
GL 5	Skin	6.31±0.10^{ef}	6.47±0.05^{d}	6.17±0.25^{e}	6.26±0.03^{e}
	Liver	5.73±0.12^{e}	5.87±0.07^{f}	5.58±0.16^{g}	5.62±0.01^{de}
	Gill	6.15±0.00^{de}	6.31±0.08^{d}	5.98±0.07^{d}	6.06±0.02^{e}
	Intestine	6.01±0.02^{abc}	6.12±0.02^{g}	5.87±0.04^{e}	5.91±0.04^{e}
GL 6	Skin	6.11±0.01^{c}	6.21±0.03^{b}	5.79±0.08^{b}	5.97±0.03^{b}
	Liver	5.54±0.03^{c}	5.67±0.05^{de}	5.18±0.02^{d}	5.41±0.03^{bc}
	Gill	6.00±0.04^{c}	6.16±0.06^{c}	5.81±0.04^{c}	5.86±0.01^{c}
	Intestine	5.89±0.09^{bcd}	5.99±0.07^{e}	5.61±0.20^{b}	5.64±0.08^{b}
GL 7	Skin	5.87±0.06^{a}	5.96±0.01^{a}	5.59±0.00^{a}	5.66±0.02^{a}
	Liver	5.34±0.04^{b}	5.55±0.02^{b}	5.07±0.01$_{b}$	5.35±0.01$_{b}$
	Gill	5.89±0.10^{b}	5.95±0.04^{a}	5.62±0.02^{a}	5.78±0.10$_{b}$
	Intestine	5.68±0.07^{ab}	5.77±0.10^{b}	5.46±0.03^{a}	5.59±0.18^{a}
OXY 8	Skin	6.33±0.00^{f}	6.46±0.10^{d}	6.17±0.07^{e}	6.29±0.06^{e}
	Liver	5.64±0.11^{d}	5.73±0.08^{e}	5.48±0.12^{f}	5.59±0.04^{d}
	Gill	6.21±0.08^{e}	6.32±0.06^{d}	6.08±0.08^{e}	6.14±0.08^{f}
	Intestine	5.98±0.04^{cd}	6.08±0.00^{fg}	5.71±0.03^{c}	5.84±0.01^{c}
OXY 9	Skin	6.26±0.02^{de}	6.36±0.04^{c}	6.13±0.00^{e}	6.18±0.02^{d}
	Liver	5.54±0.03^{c}	5.65±0.00^{d}	5.31±0.01^{e}	5.45±0.03^{c}
	Gill	6.13±0.04^{d}	6.21±0.01^{c}	5.98±0.04^{a}	6.04±0.07^{de}
	Intestine	5.78±0.01^{abc}	5.92±0.07^{d}	5.49±0.05^{a}	5.69±0.01^{a}

Mean followed by the same letter in the column were not significantly different ($p > 0.05$), DL = Drumstick Leaves, GL = Guava Leaves, OXY = Oxytetracycline

Discussion

The enterobacteriacea and total viable counts in water sample at 4 and 8 weeks were lower in treated groups when compared to the control. This study was in agreement with Bello *et al.,* (2012) and Shalaby *et al.,* (2006) who obtained decrease in bacterial loads of water fed *C. gariepinus* on walnut leaf and onion bulb and *O. niloticus* on garlic and chloramphenicol at different graded levels. However, the value of enterobacteriacea and total viable counts obtained in this study was higher than value obtained by Bello *et al.* (2012) this may be due to difference in the supplementary diets, age, size of the experimental fish used and geographical location of where the study was carried out. The results showed that enterobacteriacea counts of the skin, liver, gills and intestine was higher in control than the treated groups at 4 and 8 weeks. The values decreased in treated groups as the level of inclusion increased and as the months increased.

The total viable count (TVC) was higher in the control for skin, liver, gills and intestine of *C. gariepinus* than the treated groups at 4 and week 8 respectively. The values of decreased in treated groups as the levels of inclusion increased and as the month increased and they were significantly lower ($p<0.05$) than the control. This reduction in values of enterobacteriacea counts

and total viable counts might be due to the antimicrobial property present in *M. oleifera*, *P. guajava* leaves extract and oxytetracycline. These findings were in agreement with Bello *et al.,* (2012) who reported low value in skin, liver, gill and intestine of *C. gariepinus* fed onion bulb and walnut leaves extracts when compared to the control. Also, the report of Shalaby *et al.* (2006) was in accord with this present study who reported low values in the muscles and intestine of *O. niloticus* fed *Allium sativum* and chloramphenicol at different graded levels.

Conclusion

Based on observations of this studyon antimicrobial activities of *M. oleifera* and *P. guajava* leaves extracts, it can be concluded that these plants can be used as effective antimicrobial agents against both gram positive and gram negative bacteria. Also, considering that *M. oleifera* has the lower microbial loads and antibacterial potential from tested formulations, it is therefore recommended to be used at 3% inclusion as the antimicrobial agents in fish farming.

References

Ajaiyeoba, E. O. and Fadare, D. A. (2006). Antimicrobial potential of extracts and fractions of the African walnut (*Tetracarpidium conophorum*). *African Journal of Biotechnology*, 5(22): 2322-2325.

APHA (1985). American Public Health Association, Standard Methods for the Examination of water and wastewater, 16th edition, Washington, American Public Health Association, American Water Works Association, Water Pollution Control Federation 1278p.

Bello, O. S., Olaifa, F. E., Emikpe, B. O. and Ogunbanwo, S. T. (2012c). Effect of Walnut (*Tetracarpidium conophorum*) leaf and Onion (*Allium cepa*) Bulb residues on the tissue bacteriological changes of *Clarias gariepinus* juveniles, *Bull. Anim. Hlth. Prod. Afr.* 60(2): 205-212.

Bondad – Reantaso, M. G, Subasinghe, R. P, Arthur, J. R, Ogawa, K, Chinabut, S, Adlard, R, Tan, Z and Sheriff, M. (2005). Disease and health management in Asian Aquaculture. *Vet. Parasitol.*, 132: 249 – 272.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environ Microbiol*. 8: 1137 – 1144.

Sanjoy, B., Lee, M. K., Mohamed, S., Helen, K. and Fatima Md, Y. (2012). Antibacterial activity of neem (*Azadirachta indica*) leaves on Vibrio spp isolated from cultured shrimp. *AsianJournal of Animal and Veterinary Advances,* pp 1-7.

Shalaby A. M., Khattab Y. A. and Abdel Rahman A. M. (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia *(Oreochromis niloticus*), *J. Venom. Anim. Toxins incl. Trop. Dis,* 12 (2): 172-201.