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COMPARISON OF ANTIMICROBIAL ACTIVITIES OF BRINE SALTING, CHLORINATED SOLUTION AND *MORINGA OLEIFERA* PLANT EXTRACTS IN FISH FROM LAKE VICTORIA BASIN OF KENYA

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ABSTRACT

Chemical preservatives can be used to reduce the overall microbial populations in fish and fish products. This study was set to determine the antimicrobial activities of brine salting, chlorinated solution, and *Moringa oleifera* plant extracts treatments on enteric bacteria in *Rastrineobola argentea* and *Oreochromis niloticus* fish collected from beaches and markets in Lake Victoria basin of western Kenya. Using a cross-sectional samples collection design, fresh fish (*Rastrineobola argentea* and *Oreochromis niloticus*) samples were randomly collected from three fish landing beaches (Dunga, Luanda Rombo and Sirongo) and from three markets (Kisumu, Luanda and Bondo), in the Lake Victoria Basin of western Kenya and taken to the laboratory within 4 hours for processing and treatments with preservatives. The preservatives were sodium chloride solutions (3%, 6%, 9% and 12%), sodium hypochlorite (50ppm, 100ppm, 150ppm and 200ppm), and *Moringa oleifera* n-hexane and ethanol extract solutions (20µg/ml, 40µg/ml, 60µg/ml, and 80µg/ml), respectively. The treatments were done for 0hrs, 2hrs, 4hrs, 6hrs and 8hrs, before bacteria growth analysis using most probable number and aerobic plate count methods. Statistical differences in effectiveness of the preservatives was determined by one factor ANOVA with $P<0.05$ value considered statistically significant. The results showed that with increase in salt concentration and after 8 hours treatment duration, there was a significant reduction of microbial load in *R. argentae* and *O. niloticus* fish samples, $P<0.05$, one factor ANOVA. The effectiveness of chlorinated solution (sodium hypochlorite) against the bacteria decreased with time but increased with increase in concentration. As *M. oleifera* n-hexane and ethanol extracts concentrations increased and after 8 hours treatment duration, there was significant decrease in microbial loads, $P<0.001$ by one factor ANOVA. Overall, sodium chloride at 12% concentration was the most effective and chlorine solution even at 200ppm was the least effective while the *M. oleifera* plant extracts solutions were moderately effective against bacteria in fish. These results show that other than the traditional sodium chloride, *M. oleifera* plant extracts can also be used as antimicrobial agent for processing and preservation of fish.

Key words: enteric, preservatives, extracts, antimicrobial, effectiveness

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INTRODUCTION

Fresh fish is highly perishable, especially in tropical temperatures and need to be preserved for long- term storage. Due to the perishable nature of fish, traditional methods of preservation have been developed over the years which include salting, drying and smoking [1]. Control of pathogenic microbes by salting can be advantageous because it is non-selective and will not lead to development of microbial resistance as is common with conventional antibiotic drugs. This is because salt is chemically unrelated to the antibiotics which are used for treating microbial infections in humans and veterinary animals and can also be effective in the control of microbes, which can be mechanically transmitted by insects such as beetles, houseflies and cockroaches common in fish processing and storage areas in developing countries [2]. The preservative effect of sodium is mainly due to the decrease in water activity and thus prevention of growth of many spoilage microorganisms along with the formation of a membranous surface which further inhibits the growth of microorganisms [3, 4]. Moreover, chloride ions are toxic for some microorganisms [5].

Salting has been used for many years to preserve fish and fishery products [1, 6, 7, 8]. Salting has no adverse effects on the value of the fish protein and bacterial growth can be significantly retarded by the presence of sufficient quantities of common salt (sodium chloride). When fish is placed in a brine solution, the salt penetrates the fish and water is extracted from the tissues by osmosis. At salt concentrations of 6-10% in the fish, the activity of most bacteria that cause contamination and spoilage is inhibited [9]. Since fish contains 70-80% water, the amount of brine used must be adjusted accordingly. The higher the salt contents in the fish, the longer the shelf life [10]. Traditional methods involve rubbing salt on the flesh of the fish or making alternate layers on fish which causes the problem of un-uniform application of salt. Brining takes care of this problem, which involves immersing the fish into pre-prepared solution of salt; the advantage is that salt concentration can be more easily controlled and salt penetration is uniform [4].

Chlorinated solutions can be used as a method of sanitizing or eliminating bacteria and moulds from fish and sanitizing agents such as sodium hypochlorite have generally been proven effective in reducing overall bacterial populations as well as numbers of specific bacterial pathogens on fish and other food products [11, 12]. Chlorinated solutions have been used for sanitizing fish products especially during processing [13]. The inhibitory or lethal activity of chlorine depends on the amount of free available chlorine in the solution in the form of hypochlorous acid present in bleach and chlorine solutions that comes in contact with microbial cells. Free chlorine disinfects by chemically disrupting bacterial cell walls and membranes through oxidation of a chemical group known as the thiol group [14].

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Moringa oleifera is the best known of the thirteen species of the genus *Moringa* and the family moringaceae [15]. *M. oleifera* is a fast growing, aesthetically pleasing small tree adapted to arid and sandy conditions. The species is characterized by its long, drumstick shaped pods that contain its seeds [15]. It is currently being examined as a bio-enhancer of drugs and nutrients because of its production of compounds with antibiotic activity. The seed of *Moringa* are considered to exhibit antipyretic and antimicrobial activities, used for water purification and are also a good source of non-desiccating oil [16]. Its antibiotic property was identified as pterygospermin, a bactericidal and fungicidal compound [16, 17].

Studies have shown that an aqueous extract made from *M. oleifera* seeds is equally effective against the skin infecting bacteria *Staphylococcus aureus* as the antibiotic neomycin [18]. It has detoxifying effects which may come from Moringa's ability to purify water. It acts as a coagulant attaching itself to harmful material and bacteria by flocculating Gram–positive and Gram–negative bacterial cells [19, 20]. It is believed that this takes place in the body as well [21]. Doughari *et al.* found that 100mg/ml of *M. oleifera* plant extracts inhibit the growth of *S. typhi* [22]. Despite the use of Moringa tree, scanty literature is available on the uses of *Moringa oleifera* plant parts as sanitizers or preservatives in foods. An important step in the screening of a plant material for sanitizing/preservative activity is to evaluate its antimicrobial activity against food–borne microorganisms. The determination of a plant's antimicrobial activities against food–borne microorganisms may promote the plant to further tests geared towards its evaluation as a sanitizer or preservative in foods. It was important to evaluate the antimicrobial activities of n-hexane and ethanol seed extracts of *Moringa oleifera* against enteric bacteria in fish *Rastrineobola argentea* and *Oreochromis niloticus* as reported in this paper.

This study reports a comparison of the antimicrobial activities of brine salting, chlorinated solution and *Moringa oleifera* plant extracts treatment on enteric bacteria in *Rastrineobola argentea* and *Oreochromis niloticus* fish collected from beaches and markets in Lake Victoria Basin of western Kenya.

METHODOLOGY

Study Sites

The fish (*Rastrineobola argentea* and *Oreochromis niloticus*) samples for this study were collected from three fish landing beaches namely Dunga, Luanda Rombo and Sirongo and from three markets: Kisumu municipality, Luanda and Bondo within L. Victoria basin of western Kenya. Sirongo beach is in Bondo county; Luanda Rombo beach is in Suba county; and Dunga beach is in Kisumu county, all in the shores of Lake Victoria, Kenya. Kisumu municipality market is in Kisumu city, Winam Division of Kisumu county; Luanda market is along Kisumu-Busia road while Bondo market is in Bondo

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township, Bondo county, Nyanza province, Kenya. The two fish species *Rastrineobola argentea* and *Oreochromis niloticus* which were studied are the most commonly consumed fish species in the Lake Victoria basin region [23].

Samples Collection

Using a cross-sectional samples collection design, approximately one kilogram of fish samples, about 500-600 pieces of *R. argentea*, and whole fresh *O. niloticus*, respectively, were randomly collected from each fish-landing beach or from each market and placed in sterile plastic bags. The fresh fish samples from the fish landing beaches were collected (bought) from fishermen from different landed fishing boats, and those from the markets also collected (bought) from the fish traders. All the collected fish samples were placed in clearly labeled containers or plastic bags and then transported in cooler boxes with ice packs within four (4) hours of collection to the Maseno University School of Public Health and Community Development, Department of Biomedical Science and Technology laboratory for experiments involving treatments with different preservatives (sodium chloride, chlorinated solution and *M. oleifera* plant extracts).

Preparation of the Preservative Solutions

Commercial sodium chloride (common salt) was weighed into 3g, 6g, and 12g and dissolved in 100ml distilled water in sterile 250ml conical flasks, respectively, to make different concentrations of the brine solutions and each filtered through 0.45 micron millipore filtration units (Millipore, USA) ready for experiments. Commercial sodium hypochlorite (3.85%) was used to make various parts per million (ppm) solutions according to calculations of Orindah [24]. Briefly, 0.13ml, 0.26ml, 0.39ml, and 0.52ml of commercial sodium hypochlorite (3.85%) were mixed with 100ml distilled water in sterile 250ml conical flasks to make 50ppm, 100ppm, 150ppm and 200ppm of chlorinated solutions, filtered through 0.45 micron filter units and then the filtrates used for the experiments immediately.

Four kilograms of dry seeds of *M. oleifera* were collected from Baringo district in Kenya and brought to Maseno University, Biomedical Science and Technology laboratory. The dry seeds were aired under shade at ambient temperature on an open lab tray; this was done until a constant weight was achieved for a period of 5 days, as has been previously done for other plant materials and discolored seeds discarded [25]. Two kilograms of the seeds was ground into fine powder using Sanyo ^{TM} (Shanghai, China) electric blender and placed into closed labeled container ready for extraction with n-hexane and ethanol.

For n-hexane extraction, one kilogram fine powder and 1.5lt of n-hexane were mixed in a sterile conical flask and shaken at 120 rpm for 72hrs using Orbital shaker SOI (Stuart Scientific, UK). The supernatant was filtered using Whatman filter paper No. 91, and the filtrate rotar vaporized using Eyela rotovapourizer (Tokyo Rikakikai, Japan) to get the n-hexane extract pastes. The remaining cake from n-hexane extract was air dried at room

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temperature for 12 hours then mixed with 1.5 liter of analytical grade ethanol, and shaken at 120 rpm for 72 hours, filtered through Whatman filter paper No. 91, and rotar vaporized to make the ethanol extract paste. The n-hexane and ethanol extracts pastes were then dissolved in DMSO (1mg/5ml DMSO for n-hexane extract and 1mg/2ml DMSO for ethanol extract) to yield liquid solutions, which were further diluted in distilled water to make 20mg/100ml, 40mg/100ml, 60mg/100ml and 80mg/100ml solutions (that is, 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml final concentrations) for the experiments.

Samples Processing, Preservatives Treatments and Bacterial Analysis

The fresh fish samples from the field were unpacked and coded for laboratory analysis. About ten grams of fish samples (either 10-15 whole pieces of *R. argentea*, or cut muscles with skin from lateral lines of *O. niloticus*) were used for analysis. The fish samples were macerated for 3 min in a blender (Sanyo ^{TM}, Shanghai, China) to make slurry. The resultant slurry was respectively transferred into sterile labeled 250ml flasks in readiness for preservative treatments with the already prepared 100mls of different sodium chloride solutions: 3%, 6%, 9% and 12%; sodium hypochlorite solutions: 50ppm, 100ppm, 150ppm and 200ppm; and *M. oleifera* n-hexane and ethanol extract solutions: 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml, respectively, before bacteriological analysis. In all, the five (5) fish slurry for either *Rastrineobola argentea* or *Oreochromis niloticus* in well labeled 250ml flasks, according to the treatment solution and concentrations including the control flasks, were made in readiness for the treatments and bacterial analysis experiments. Each prepared preservative solution was then added to the respective appropriately labeled 250ml flask containing the respective fish slurry up to 100ml mark, mixed thoroughly, and timed for the 0hrs, 2hrs, 4hrs, 6hrs and 8hrs experimental treatment durations. Double distilled water was used as control for all the treatment experiments.

Bacterial analysis from the various fish/preservative treatment flasks was done using most probable number (MPN) of microbe determination method [26]. Approximately 10mls of phenol red lactose broth (HiMedia Lab. Pvt. Mumbai, India) was added into each of 3 sets of 25ml tubes (with inverted Durham's tubes' inserts). Each set contained three tubes (9 tubes in total). The loaded tubes were sterilized by autoclaving. The tubes were allowed to cool and then inoculated with a ten-fold difference in respective fish/preservative treatment mixture (from the 250ml flasks), 0.1ml, 1ml, and 10ml per tube and incubated at 37^0C (Gallenkemp, Germany) to determine the number of coliforms in the respective fish/preservative treatments, with the end point determined from the most probable number McCardys MPN table [27].

Total viable bacterial counts from the fish preservative treatment experiments was also done using the aerobic plate count method which employs plate count agar according to AOAC method 966.23 with colony forming units (CFU) determination [28]. All the tests

were done in triplicate. Briefly, using a micropipette and sterile tips, aliquots of 1 ml (or 1000µl) of the various fish/preservative treatments (from the 250 ml flasks), were aseptically inoculated into sterile test tubes containing 9mls of molten plate count agar at approximately 35-40^{0}C, vortexed, and then plated into sterile Petri dishes and allowed to set for about 15 minutes. The loaded Petri dishes were then inverted and incubated at 37^{0}C (Gallenkamp, Germany) for 24 hrs. By using Quebec colony counter, the number of colonies per plate (that is, colony forming units, CFUs) was counted, and the means from the triplicate experiments for each fish/preservative treatment determined and recorded. The recorded mean values were multiplied by 10 to take care of the dilution factor of 1:10 to express the final results as CFUs per gram of fish sample.

Date Analysis and Presentation

Data entry and analysis was done using Windows Excel 2003. The fish/preservative treatments and microbiological data (MPN and CFU) from the three fish-landing beaches (Dunga, Luanda Rombo and Sirongo) and three markets (Kisumu municipality, Luanda and Bondo) were used in the final results presentation and statistical analyses. Only the means of 0 hour and after 8 hours treatment duration experiments for the various preservative concentrations and controls were compared for the two fish species. One factor analysis of variance (ANOVA) was used for determining the effectiveness of various preservative concentrations, with $p<0.05$ taken as statistically significant. The results of MPN and CFU determination for comparison of effectiveness of different preservatives, that is salt solution (12%), chlorinated solution (200ppm), *M. oleifera* n-hexane 80 µg/ml and *M. oleifera* ethanol 80 µg/ml extracts, was done by one way ANOVA and tabulated, and only the means of the 8hrs treatment duration data used.

RESULTS

With increase in salt concentration and time, there was a high reduction of bacterial load on *Oreochromis niloticus* and *Rastrineobola argentae* samples, Figure 1. At time 0h and control of 0% salt concentration the bacterial load on tilapia was 205.7 MPN/g, and at 0h and salt concentration of 12%, bacterial load decreased to 125.2 MPN/g, P=0.04 one factor ANOVA. At 8h and control (0%) salt concentration, the bacterial load was 362.2 MPN/g and at salt concentration of 12% and after 8h, the microbial load decreased significantly to 65.7 MPN/g, P=0.00002, one factor ANOVA.

In the case of dagaa, at 0h the control (0% salt concentration) had a microbial load of 241.6 MPN/g and this decreased significantly to 140 MPN/g at 0h and 12% salt concentration, P=0.007. At 8h, the control (0% salt concentration) recorded 536.6 MPN/g bacterial load and at 8h and 12% salt concentration, the microbial load decreased to 74.5 MPN/g, P=0.00001, one factor ANOVA.

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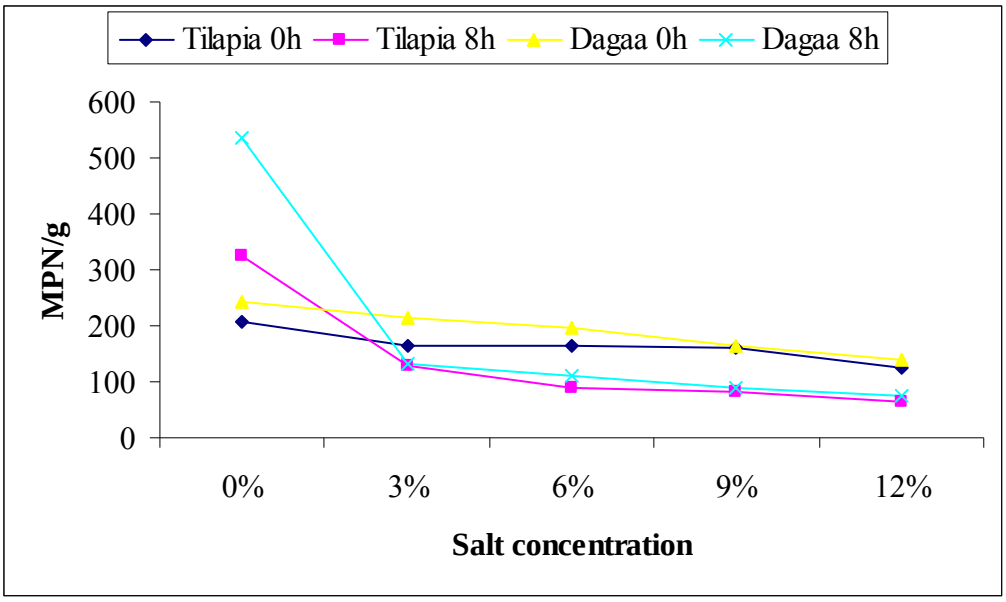

Figure 1: Antibacterial activities of salt concentrations on bacteria in *O. niloticus* and *R. argentea* fish samples at 0 hour and after 8 hours of treatment duration

The effectiveness of chlorinated solution on bacteria in *O. niloticus* and *R. argentea* fish samples decreased with time but increased with increase in concentrations, Figure 2. The microbial load in tilapia at 0h was 205.7 MPN/g at 0ppm and at 0h and 200ppm it decreased to 72 MPN/g, P= 0.00001, one factor ANOVA.

At 8h the control (0ppm) chlorine concentration, the bacterial load was 536.6 MPN/g and at 200ppm chlorine concentration, the microbial load decreased significantly to 151.5 MPN/g, P=0.00001, one factor ANOVA.

In dagaa samples, microbial load at 0h was 241.7 MPN/g at control 0ppm and this decreased at 0h at high concentration of chlorine (200ppm) to111.5 MPN/g, P=0.0002, one factor ANOVA. At 8h, the bacterial load on dagaa at 0ppm was 536.6 MPN/g and at high chlorine concentration of 200ppm at 8h it reduced to 151.5 MPN/g, P=0.00001.

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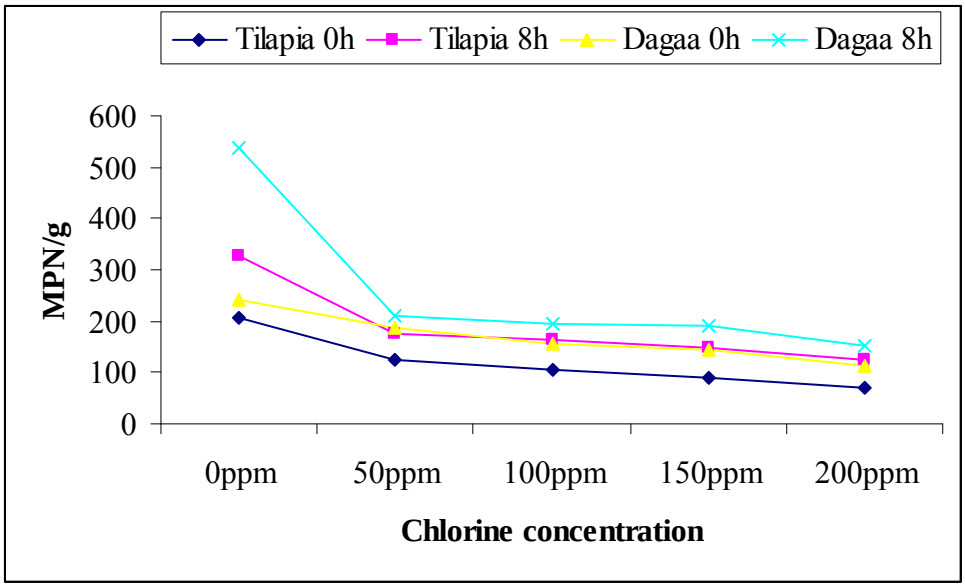

Figure 2: Antibacterial activities of chlorinated solution concentrations in *O. niloticus* and *R. argentea* fish samples at 0 hour and after 8 hours of treatment duration

As *Moringa oleifera* n-hexane extract concentration and time increased, there was decrease in bacterial load in *O. niloticus* and *R. argentea* fish samples, Figure 3. In tilapia control, microbial load at 0h was 205.8 MPN/g at 0µg/ml and this decreased to 148.3 MPN/g at a concentration of 80µg/ml, P= 0.002. At 8h the control tilapia (0µg/ml) had a microbial load of 326.2 MPN/g and at 80µg/ml it decreased to 87 MPN/g, P=0.00001. For the dagaa microbial load at 0h was 241.7 MPN/g at 0µg/ml and this decreased at 0h to 189.1 MPN/g at a concentration of 80µg/ml, P=0.5, which was less significant. At 8h, microbial load was 536.6 MPN/g 0µg/ml and this decreased to 120.8 MPN/g at 80µg/ml at 8h, P=0.00001, one factor ANOVA.

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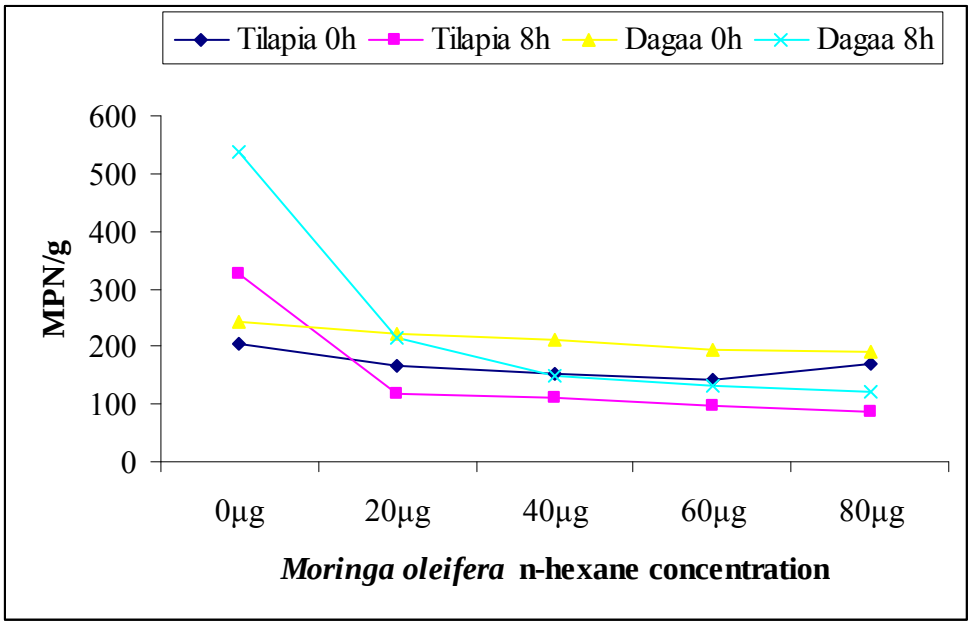

Figure 3: Antibacterial activities of *M. oleifera* n-hexane extract concentrations in *O. niloticus* and *R. argentea* fish samples at 0 hour and after 8 hours of treatment duration

As *Moringa oleifera* ethanol extract concentration and time increased, there was decrease in bacterial load in *O. niloticus* and *R. argentea* fish samples, Figure 4. In tilapia control (0µg *M. oleifera* concentration), microbial load at 0h was 205.8 MPN/g and this decreased though not significantly to 169.7 MPN/g at *M. oleifera* ethanol extract concentration of 80µg/ml at 0h, P=0.4. At 8hr control tilapia (0µg *M. oleifera*) had a microbial load of 326.3 MPN/g which decreased to 114.7 MPN/g at *M. oleifera* ethanol extract concentration of 80 µg/ml at 8h, P=0.00003. For dagaa, microbial load at 0h was 241.7 MPN/g at 0µg and this decreased to 196.7 MPN/g *M. oleifera* ethanol extract of 80 µg/ml at 0h, P=0.06. At 8h, microbial load was 536.6 MPN/g at control 0µg and this decreased to 144.3 MPN/g at *M. oleifera* ethanol extract concentration at 80µg/ml at 8h, P= 0.00001, one factor ANOVA.

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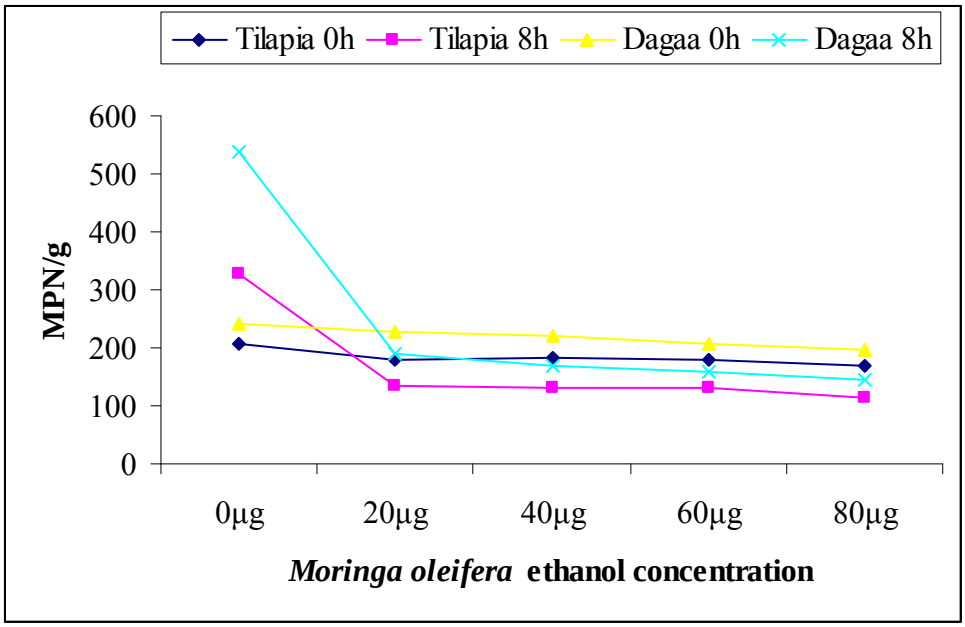

Figure 4: Antibacterial activities of *M. oleifera* ethanol extract concentrations on bacteria in *O. niloticus* and *R. argentea* fish samples at 0 hour and after 8 hours of treatment duration

As shown in Table 1 for the tilapia fish, 12% of sodium chloride solution was the most effective in reducing bacterial loads (mean of 65.5 MPN/g), then 80µg/ml of *M. oleifera* n-hexane and ethanol extracts (means of 87.6 MPN/g, and 113.6 MPN/g, respectively), while sodium hypochlorite solutions, even at 200ppm, was the least effective (mean of 123.7 MPN/g), after 8hrs of treatment, P=0.0009, one factor ANOVA. On the other hand in the dagaa fish, 12% sodium chloride solution was more effective with a mean of 74.6 MPN/g, followed by 80µg/ml of *M. oleifera* n-hexane and *M. oleifera* ethanol extracts each with 120.9 MPN/g and 143.8 MPN/g, respectively. Sodium hypochlorite solution, even at 200ppm was the least effective with a mean of 151.6 MPN/g. These differences were also significant P=0.01, one factor ANOVA.

Table 2 shows similar results for the two fish species. In the case of tilapia, 12% of sodium chloride solution was also the most effective in reducing bacterial load (mean of 39.1 CFU/g), then 80 µg/ml *M. oleifera* n-hexane and ethanol extracts (mean of 87.4 CFU/g and mean of 91.5 CFU/g), respectively, while sodium hypochlorite solution, even at 200ppm, was also the least effective (mean of 101.7 CFU/g). For the dagaa fish, 12% of sodium chloride solution was the most effective with a mean bacterial load reduction to 59.9 CFU/g, followed by 80 µg/ml *M. oleifera* n-hexane and ethanol extracts with a mean bacterial load of 95.4 CFU/g and 106.2 CFU/g), respectively. Again the least

effective preservative was sodium hypochlorite solution even at a high concentration of 200ppm with a mean bacterial load of 103.6 CFU/g, P = 0.02, one factor ANOVA.

DISCUSSION

The results from this study have shown significant effectiveness of increased sodium chloride concentration (12%) at reducing bacterial load in *R. argentea* and *O. niloticus*. These results are consistent with traditional known antibacterial effects of common salt for preserving food. Common salt preserves food in different ways but mainly by inhibiting bacterial growth through dehydration, chloride ion effect, oxygen removal, and carbon dioxide sanitization of growth of preolytic enzyme. At a concentration of ≥3% (w/v), NaCl generally inhibits the growth of *Salmonellae* [29]. The preservative effect of salt is mainly due to the decrease in water activity and thus prevention of growth of many spoilage micro-organisms along with formation of a more membranous surface which further inhibits the growth of micro-organisms [8]. Even at a low concentration of salt 3%, effect of salt on bacterial load could be observed in this study. According to Ofulla *et al.* [30] salt solution gave the best results and had a significant effect on the bacterial load on fish.

Results from this study shows that the higher the concentration of sodium hypochlorite solution, the more effective it was in inhibiting bacterial growth in fish. However, with increase in time, the less effective it became. This may be due to the amount of organic materials (fats and proteins), in fish tissues which combine with chlorine ions rather than with bacteria [31]. Sodium hypochlorite solution also gets oxidized and looses strength with time [31]. Though chlorine is always reported to be highly effective and inexpensive solution used in the control of food-borne diseases, especially during food processing, this study found that it was not effectives in reducing bacterial load in fish samples and therefore may not be recommended for treating fish for long term storage.

Higher concentrations of *Moringa oleifera* n-hexane or ethanol extracts were effective in reducing bacterial loads as found in this study. These results confirm that *M. oleifera* has antibacterial activity and hence it can be used for fish preservation. The compound pterygospermin in *M. oleifera* seeds have antibiotic property which enhance the elimination of bacteria [17]. Further, it has been also used in the coagulation and purification of water [32], meaning that it can be used as a general antibiotic and water purification in fish ponds and even aquaria.

The results of this study show that for the two fish species *R. argentea* and *O. niloticus*, 12% sodium chloride solution was the most effective in reducing bacterial load, compared to chlorinated solution even at the highest concentration of 200ppm. These results are encouraging because common salt (sodium chloride) is cheap and easily available and should, therefore, be adopted by our fisher community for routine

processing and preservation of fish. It is also encouraging that salt has no effect on the value of fish protein and when used for preserving fish even at a higher concentration, recipes for desalting can be adopted during fish preparation and cooking for human consumption which is a common and established culinary practice all over the world.

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The results also show the effectiveness of *M. oleifera* plant extracts against bacteria in fish samples, which is new information. This means that *M. oleifera* plants which easily grows in most arid and sandy conditions as found near the Lake Victoria basin of Kenya, can be exploited to provide extracts for preserving fish for long term storage and safety for human populations.

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Table 1: Comparison of antibacterial activities using MPN method of different preservatives on enteric bacteria in *Rastrineobola argentea* and *Oreochromis niloticus* fish samples after 8 hours of treatment

Preservatives (concentration)	Antibacterial activity in MPN/g		
	R. argentea	***O. niloticus***	**Mean**
Sodium chloride (12%)	74.6	65.5	70.1
Chlorinated solution (200ppm)	151.6	123.9	137.8
M. oleifera n-hexane extract (80 µg/ml)	120.9	87.6	104.3
M. oleifera ethanol extract (80 µg/ml)	143.8	113.6	128.7

Table 2: Comparison of antibacterial activities using CFU method of different preservatives on enteric bacteria in *Rastrineobola argentea* and *Oreochromis niloticus* fish samples after 8 hours of treatment

Preservatives (concentrations)	Antibacterial activity in CFU/g		
	R. argentea	***O. niloticus***	**Mean**
Sodium chloride (12%)	59.9	39.1	49.5
Chlorinated solution (200ppm)	103.6	101.7	102.7
M. oleifera n-hexane extract (80 µg/ml)	95.4	87.4	91.4
M. oleifera ethanol extract (80 µg/ml)	106.2	91.5	98.9

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