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Closed Rank Effects of Ginger Oil and Moringa Oil on Bacteria Isolates of African Catfish (*Clarias gariepinus*)

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Abstract: The antimicrobial activity of bacteria isolated from the skin, gills and intestine of *Clarias gariepinus* was carried out using *Zingiber officinale* (Ginger) and *Moringa oleifera* (Moringa) oil extracts. The lowest and highest bacteria count were (3.0×10^5) and (3.3×10^6) respectively. The least value was observed from the skin and the highest from the gills. Morphological and biochemical characteristics of isolates revealed presence of bacteria species such *Salmonella* spp., *klebsiella* spp., *Pseudomonas* spp., *Escherichia coli* and *Staphylococcus aureus*. The bacteria isolates were sensitive to Ginger and Moringa both individually and in combination while using the disk diffusion method which showed the inhibition zone (3mm-16mm) from day 1 to day 2 for all isolates. *Salmonella* spp. was the most effective having a mean value of (15.33±0.58) and *E. coli* was being the most resistance with a mean value of (3.6±0.58) for all extracts. Results showed that both Ginger and Moringa extracts are not significantly different from each other at ($P>0.05$) but are both significantly difference at ($P<0.05$) when compared with the control. The results of the study confirmed the Ginger and Moringa were more effective individually than when combined. The study therefore suggests that Medicinal plant extracts such as ginger and moringa should be used as alternative method as its action is indicative in preventing emergence of resistant bacteria and improving the antimicrobial role as well as the quality of fish.

Key words: Ginger · Moringa · *Clarias geriepinus* · Bacteria

INTRODUCTION

Fish culture is a relatively new enterprise in the developing world especially in Africa. All fish live in microbe rich environment and are vulnerable to invasion by pathogenic and opportunistic microorganisms. The environment of fish being aquatic is very challenging with fish in constant interaction with a wide range of pathogenic and non pathogenic microorganisms [1].

Bacteria agents are among the highly encountered causes of diseases in stressed warm water agriculture. The presence of bacteria in fish could play diverse some of which might be beneficial to the fish itself. However, the presence of some bacteria species could lead to post harvest spoilage and adverse health conditions.

Environmental degradation due to pollution of natural water bodies and poor culture conditions of some culture fish ponds have elicited the presence of these pathogenic agents [2] with bacteria being one of the most common microorganisms of farmed catfishes. Fishes are sometimes overwhelmed by those pathogenic agents and succumb to infections.

Clarias gariepinus is a popular fish for aquaculture because of the hardness of larval production in captivity and good market price. Investigations have been conducted on the bacteria infecting catfish.

Aquatic bacteria that infect fish belong to three groups, the Gram-negative bacteria (Most common), Gram-positive bacteria and Acid-fast bacteria, which are obtained from food or from the environment Gram-negative bacteria, cause most diseases in a tropical fish. Several workers have conducted investigation on these bacteria [3]. Some of which are opportunistic pathogens while others are obligatory pathogens. For example, *Escherichia coli* area gram negative bacillus which causes serious infectious diseases such as urinary tract infection and blood stream infection.

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Salmonella typhimurium, a Gram-negative bacterium is recognized as one of the leading cause of food borne bacteria diseases which most people infected with salmonella develop diarrhea, fever, vomiting and abdominal cramps during 8hrs after infection. Many problems of human salmonellas are following consumption of contaminated foods having increased dramatically worldwide.

Moreover, *Staphylococcus aureus* is one of the most common gram positive bacterium that causessevere, nausea, abdominal cramps, vomiting and diarrhea.

In an attempt to improve fish health by using biological agents to prevent infection rather than using drugs as cure, medicinal plants are considered.

Ginger plant is a spice that has been noted for its medicinal values on an analgesic, sedative, antipyretic and antibacterial agent [4]. Gingeralso has Anti-microbial, anti-oxidative and seasonal qualities.

The moringa tree is a native of north eastern India which is rich in nutrients used for both industrial and medicinal applications.

The aim of this study was to examine the synergistic effect of ginger and moringa oil extract as anti-microbial agents on the skin, gills and intestinal bacterial isolates of African catfish; *Clarias gariepinus*.

MATERIALS AND METHODS

Collection of Sample and Bacterial Isolation: Skin of fish was aseptically removed and body dissected to get the gills and intestines. Serial dilution was done in 5 fold each for the 5 samples.25 test tubes were labeled and kept in test tube rack. Then 9ml of sterile saline was put into each test tube (Per each labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}). The test tubes with saline were kept in an autoclave per 15minutes. After cooling one gram of each was weighed and put in saline in 10^{-1} test tube, then a sterilized pipette was used to introduce 1ml of each sample after vigorous shaking into others (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) aseptically. The flames from Bunsen burner was used throughout this experiment under laminar air flow to enable proper sterility. After several dilutions, a micropipette was used to introduce 1ml of each sample of the suspension into both nutrient and Mackonkey agar respectively. It was spreaded with glass spreading rod under sterile condition and allowed to dry at room temperature per 10 minutes. All the plates were incubated at 37°C for 24hrs. After incubation, the numbers of colony forming unit (CFu) between 30 and 300 were recorded and bacterial densities expressed in cfu/ml of water samples. The bacteria colonies that grow on the media were selected and subcultured using nutrient and Mackonkey to be purified and subjected to morphological and biochemical test for identification.

Identification of Bacteria Isolates: Using gram staining technique and biochemical characterization according to [5] bacteria identified include *Staphylococcus aureus, Salmonella* spp., *Klebsiella* spp. *Pseudomonas* spp. and *Escherichia coli.*

Preparation of Plant Extract: Peels of ginger were removed cut into pieces and grounded with blender then oven dried at 60°C for 1hr while moringa seed were also removed from its shell grounded and over dried at 60°C for 30mins.

Samples were then prepared for extraction using Soxhlet extraction with using a non-polar solvent (n-hexane), 300ml for every extraction with 200g of sample. The water in the condenser boils until 100°C. The water solvent mixes with the extract, therefore no vaporization. This process is called siphoning. The solvent was eventually removed from the extract in hot air oven to leave pure oil. Extracts were stored at 4°C and were used to prepare the antibiotic disc per the antimicrobial study.

Preparation of Plate Innoculum: Sterile swab stick was used to pick pure bacteria isolates and then used to inoculate a Mueller-hinton agarplates by streaking and ensuring even distribution of the innoculum. This was done for each bacteria isolates.

Antimicrobial Inhibition: Disk diffusion test was performed using standard procedure adopted by Kirby – Bayer disk diffusion method. Sterile 6mm filter paper disc containing 100% concentration of Ginger and Moringa oil extract respectively and mixture of both (i.e. 50% of Ginger and Moringa oil extract) were aseptically placed on the already inoculated Mueller hinton agar plate. Also, aliquot of distilled water was added to a sterile disc as control. The plates were incubated at 37°C for 24-48hrs to validate for the inhibition zone. Diameters of inhibition zones were measured to the nearest millimeters. Each experiment was done in triplicate.

A clear zone of inhibition (Plaque) indicates absence of bacteria growth while no discernable plaque around the disc means that the bacteria are growing normally. The presence of plaque means sensitivity while its absence means resistance.

RESULTS

There were differences in the microbial load of skin, gills and intestine of fish samples with the least count of 3.0 x 10^3 and highest figure of 3.3 x10^6. From the result skin has the lowest microbial count and the gills have the highest count as seen in Table 1.

Table 2 indicates that the organism isolated from *Clarias gariepinus* reacted both positive and negative to Gram reaction but having more of Gram-negative organism.

According to the biochemical test conducted on each organism *Salmonella* spp. reacted positive to motility test, positive to hydrogen sulphide test and gas was produced, using carbohydrate. Fermentation test, it was positive to lactose and negative to glucose, while test on catalase, coagulase, indole, urease, citrate and oxidase remain negative, it also reacted negatively to gram reaction.

For *Escherichia coli* both glucose and lactose were positive and gas was produced while hydrogen, sulphide, catalase, coagulase, indole, motility and oxidase negative.

Gram reaction is also negative pseudomonas reacted negativity to gram reaction, negative to catalase, coagulase, indole, urease, oxidase but positive to citrate, motility, glucose, lactose, hydrogen, sulphide and has product.

Staphylococcus aureus is the only specie that reacted positive to gram reactions, both catalase and coagulase test were positive while glucose, lactose, hydrogen, sulphide, motility, indole, urease, citrate and oxidase are negative and gas was not produced as well.

The obtained results of disk diffusion test according to Table 3 indicated that oils extracts from ginger and moringa seed showed different rate of inhibition growth depending on the bacterial strains.

Diameter of inhibition zone for *Staphylococcus aureus* shows relatively the same in ginger and moringa oil extract both individually and when combinedwhile there is a higher sensitivity rate for moringa than ginger in *E. coli* and quite lower when combined. Same goes for *klebsiella* and *Pseudomonas*.

Salmonella showed the highest zone of inhibition for ginger and moringa oil and the combination as well indicating to more sensitive to the extracts and *E. coli* more resistance having the least value 1

There is a zero or one control effect on all isolated organism.

According to the Table 4, there is a significant different in the values of inhibition zone for all the organisms against the extract in each day except for salmonella on ginger oil extract which has 15.30±0.58 for both day 1 and 2. Salmonella has the highest inhibition zone per both Ginger and Moringa and when combined as well but individually. Pseudomonas has the least value of 4.67±0.58 in day 1 and 5.33±0.58 in day 2 per ginger oil extract. *E coli* has the least value of 7.0±10 in day and 8.0±1.0 in day 2 per moringa oil extract, *E. coli* also has the least value of 3.67±0.58 in day 1 and 4.0±1.00 in day when both Ginger and moringa oil extract were combined. The control has the least significance in *klebsiella* with 0.0±0.00 in day 1 and 0.33±0.58 in day 2.

According to Table 5, there was no statistically significant relationships between ginger oil extract, moringa oil extract and ginger and moringa oil extract for all the bacteria. But, it is conspicuously clear and established that there were statistical significant association between the controls and all the extract for all bacteria.

DISCUSSION

Fish is a low acid food that can readily support the growth and pathogens particularly bacteria if not properly handled and rapidly processed after harvesting. This partly explains why despite the fact that all the fish samples shows growth of heterotrophic bacteria and fungi, the bacteria load was consistently higher than the fungal load.

The concentration of bacteria associated with fish samples in this study is high; this high level of bacteria calls for concern and provides an early warning. Since the fishes reared in them stand the potential risk of being devastated by disease outbreak with time if the level is not monitored.

In this study, bacteria were recovered from the gills, skin and intestine of *Clarias gariepinus*. The highest bacteria count was detected in the gills (3.3 x 10^6cfu/ml). This confirmed the findings of [7], that fish take a large number of bacteria into their gills from the water sediment. The lowest bacteria count was discovered in the skin (3.0 x 10^5cfu/ml).

The isolated organisms were more of gram-negative bacteria than gram positive bacteria. In the previous study of [3], the gram negative bacteria species isolated from the body surface of *Clarias gariepinus* in Nigeria were those in the genera *Pseudomonas*, *Serratia*, *Enterobacter* and *Escherichia* while in this current study similar genera of gram-negative bacteria species were isolated from the skin, gills and intestine of *Clarias gariepinus*.

Table 1: Total colony forming unit of bacteria isolated from skin, gills and intestine of *Clarias gariepinus*

Fish	Skin (cfu/ml)	Gills (cfu/ml)	Intestine (cfu/ml)
1	3.2×10^5	2.0×10^6	4.4×10^5
2	3.0×10^5	1.8×10^6	3.1×10^5
3	3.8×10^5	1.3×10^6	4.8×10^5
4	4.1×10^5	3.3×10^6	5.5×10^5
5	3.5×10^5	1.6×10^6	4.6×10^5

Table 2: Morphological and Biochemical Identification of organism isolated from *Clarias gariepinus*

Fish	Gram reaction	Slant Glucose	Butt lactose	H_2S	Gas	Catalase	Coagulase	Motility	Indole	Urease	Citrate	Oxidase	Organism
Gills	GNR	+	+	-	+	-	-	+	-	-	-	-	*Escherichia Coli*
	GNR	-	+	+	+	-	-	+	-	-	-	-	*Salmonella* spp.
	GNR	+	+	-	+	-	-	-	-	+	+	-	*Klebsiella* spp.
	GNR	+	+	+	+	-	-	+	-	-	+	-	*Pseudomonas* spp.
	GPC	-	-	-	-	+	+	-	-	-	-	-	*Staphylococcus aureus*
Intestine	GNR	+	+	-	+	-	-	-	-	+	+	-	*Klebsiella* spp.
	GNR	-	+	+	+	-	-	+	-	-	-	-	*Salmonella* spp.
	GNR	+	+	-	+	-	-	+	-	-	-	-	*Escherichia Coli*
	GNR	+	+	+	+	-	-	+	-	-	+	-	*Pseudomonas* spp.
	GPC	-	-	-	-	+	+	-	-	-	-	-	*Staphylococcus aureus*
Skin	GNR	+	+	+	+	-	-	+	-	-	+	-	*Pseudomonas* spp.
	GPC	-	-	-	-	=	+	-	-	-	-	-	*Staphylococcus aureus*
	GNR	+	+	-	+	-	-	+	-	-	-	-	*Escherichia Coli*

Table 3: Anti-bacterial activity of ginger and moringa oil extracts on bacteria isolated from *Clarias gariepinus* using disk diffusion test.

	Bacteria	Incubation day	Ginger oil extract			Moringa oil extract			Ginger & moringa extract			Control		
1	*Staphylococcus aureus*	1	8	9	9	8	8	9	7	8	9	0	1	0
		2	10	10	10	9	10	11	8	10	10	0	1	1
2	*Escherichia coli*	1	6	5	4	8	6	7	4	3	4	0	0	1
		2	6	5	5	9	7	8	4	3	5	1	0	1
3	*Salmonella* spp.	1	15	16	15	14	14	13	15	15	16	1	0	0
		2	15	16	15	15	15	14	16	15	16	1	1	0
4.	*Klebsiella* spp.	1	5	6	6	10	10	11	6	7	6	0	0	0
		2	6	6	7	11	11	12	7	8	8	0	0	1
5.	*Pseudomonas* spp.	1	5	5	4	11	12	12	6	6	5	0	0	1
		2	5	6	5	12	14	13	7	7	6	1	0	1

Table 4: Final results of organism against ginger and moringa oil extract

Organism	Day	Ginger oil	Moringa oil	Ginger & Moringa oil	Control
Staph aureus	1	8.67 ± 0.58	8.33 ± 0.58	8.0 ± 1.00	0.33 ± 0.58
	2	10.0 ± 0.0	10 ± 1.0	9.33 ± 1.15	0.67 ± 0.58
E. Coli	1	5.0 ± 1.0	7.0 ±1.0	3.67 ±0.58	0.33 ± 0.58
	2	6.34 ± 0.58	8.0 ± 1.0	4.0 ± 1.00	0.67 ± 0.58
Salmonella	1	15.33 ±0.58	13.67 ± 0.58	15.33 ± 0.58	0.33 ± 0.58
	2	15.33 ± 0.58	11.67 ± 0.58	15.67 ± 0.58	0.67 ± 0.58
Klebsella	1	5.67 ± 0.58	10.33 ± 0.58	6.33 ±0.58	0.00 ± 0.00
	2	6.33 ± 0.58	11.33 ± 0.58	7.67 ± 0.58	0.33 ± 0.58
Pseudomonas	1	4.67 ± 0.58	1.67 ± 0.58	5.67 ± 0.58	0.63 ± 0.58
	2	5.33 ± 0.58	13.0 ± 1.0	6.67 ± 0.58	0.67 ± 0.58

Values are represented as mean ±standard deviation

Table 5: T-Test, two sample assuming unequal variances

		Sample		Probability
1.	Ginger oil	Moringa oil	P>0.05	Non significant
2.	Ginger oil	Ginger and moringa oil	P>0.05	Non significant
3.	Ginger oil	Control	P<0.05	Significant
4.	Moringa oil	Control	P<0.05	Significant
5.	Moringa oil	Ginger and moringa oil	P>0.05	Non significant
6.	Ginger and Moringa oil	Control	P<0.05	Significant

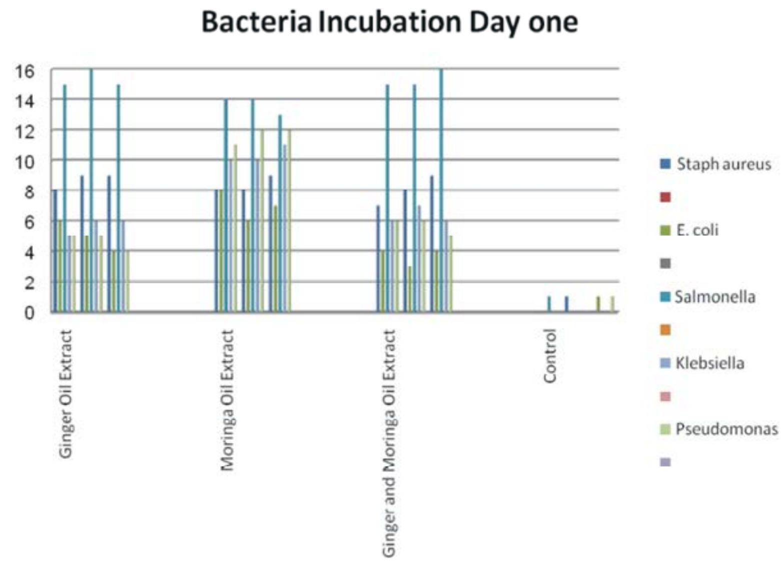

Fig. 1: The zoning diameter at day 1

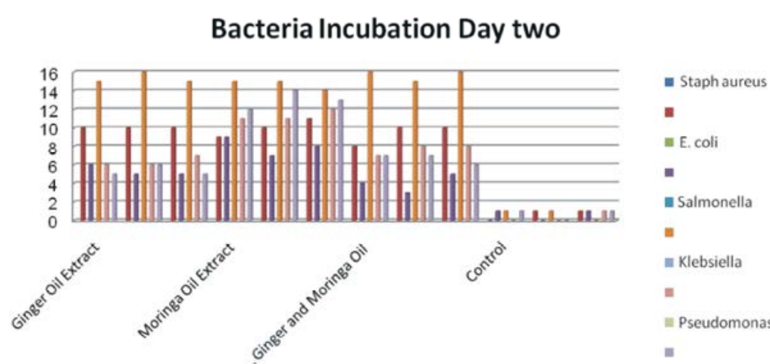

Fig. 2: The zoning diameter at day 2

The bacteria identified according to Table 2 comprised mainly of normal flora of fish ponds, skin gills and intestine of fish processes and these include members of the genera *Escherichia, Salmonella, Klebsiella, Pseudomonas* and *Staphylococcus*.

Staphylococcus aureus species is a normal flow of many water bodies and the skin of the fish handlers. The finding is in agreement with that of [8] who reported a 44.6% prevalence rate per S*taphylococcus aureus* in smoked catfish and tilapia sold in Jos, Nigeria.

Also, this result is in line with the findings of [9] who gave similar report of the occurrence of *E. coli* and *Pseudomonas* spp. in fish. *E. coli* and *Pseudomonas* spp. are pathogens that can cause intestinal infections and nosocomal infections respectively in humans. It is however noteworthy that contrary to the reports of [8], the common human pathogen, *Listeria monocytogenes* was not detected in the fish samples.

Different methods are available for reducing the population of fish pathogens in aquaculture. Though bacteria diseases on commercial fish farms are currently treated with antibiotics, this form of treatment has clinical implications resulting in the emergence of some resistant flora that can infect humans via the food chain, thereby providing risks to consumer health. Moreso, it has been identified that there is emergence of antimicrobial resistance following the use of antimicrobial agents in aquaculture [10, 11].

A growing concern for the high consumption of antibiotics in aquaculture has therefore initiated a search for alternative methods of fish diseases control.

The traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. It is important to investigate scientifically those plants which have been used in traditional medicine as potential sources of novel antimicrobial compound.

For this study, ginger and moringa oil extracts were considered. All the 5 bacteria isolated from *Clarias gariepinus*were sensitive to ginger and moringa oil extracts according to Table 3. Four gram-negative bacteria (*Salmonella* spp., *E. coli, Klebsiella* spp. and *Pseudomonas* spp.) and one gram positive (*Staphylococcus aureus*) were inhibited by the extracts. The observation of both Gram-negative and gram-positive effects in the same plant extract may be explained by the presence of a wide spectrum of bactericidal substances or by the action of toxins produced by the plant [12].

Salmonella spp. has the highest inhibition zone with diameter ranging from (13-15mm) per ginger, moringa and combination of the two.

Escherichia coli is the least effective giving the inhibition zone ranging from (3-9mm) for all extracts.

For ginger extract, it was more sensitive for *Salmonella* spp. (15-16mm) while the least effective is *Pseudomonas* spp. (4-6mm).

If also supports the work of [13] that ethanol extract of ginger produced higher inhibition zone than the garlic extract. It also add ginger to the list of potential plant materials processing inhibitory property against typhoid fever organism of which *Salmonella typhi* is known to cause typhoid fever.

The result support [14] that affirmed that the extraction of ginger by using either methanol or n-hexane has an antimicrobial activity against both bacteria and fungi and this may be caused as a result of the presence of gingerol and shogaol as active ingredients within ginger.

The result also support the activity of [15] who reported the antimicrobial activity of ginger plant extracts had microbial inhibition activities towards *E. coli, S. anteric, S. aureus* and *V. parahoemplyticus*.

In addition [16] who worked on the effects of different concentrations of ginger on smoked dried *Clarias gariepinus*.

The antimicrobial action of ginger has also been reported by [17] using ginger in combination with honey against staphylococcus isolates.

Ginger definitely has antimicrobial properties and its use in the prevention of emergence of resistant bacteria is of high benefit.

Like the ginger oil salmonella was the most effective for moringa oil extract ranging from (15-16mm) while the least effective was *E. coli* (6-9mm).

However, the observation is in line with the report of [16] who showed activity of *Moringa oleifera* hexane extract against *Salmonella, Shigella* and *Enterobacter*.

The result also corroborated by the report of [18], that ethanol extract of moringa displayed bioactivity on pathogenic *Aeruginosa, Salmonella typhi* and *Enterobacteraerogenes*.

The report supports the findings of [19] indicating that the treatment of fish with *Moringa oleifera* extract as used in this study is useful in the elimination of *Escherichia coli*.

It has been suggested that Moringa contain bio-components whose antibacterial potentials are highly comparable with that of the antibiotic tetracycline against all Gram-negative and Gram-positive bacteria tested [20].

The aqueous seed extract of *Moringa oleifera* reversed high level of phytochemicals such as saponins, alkaloids, cardiac glycosides, flavonoids and anthraquinones as reported by [21].

The fractionated leaves portion revealed low phytochemical contents when compared to that in *Moringa oleifera* seed extract. The *Moringa oleifera* seed extract revealed the presence of free anthraquinones and cardiac glycosides which is in contrast with the findings of other researchers who worked on different parts of moringa.

The aqueous seed extract of *Moringa oleifera* used in this experiment was found to be inhibitory on all the pathogenic bacteria isolated from *Clarias gariepinus* which include *Staphylococcus aureus, Escherichiacoli, Pseudomonas* spp., *Klebsiella* spp. and *Salmonella* spp. [4] reported similar activity though in their research, no activity was reported on *Klebsiella* spp.

The antibacterial activity exhibited on the bacteria isolates could be as a result of the presence of flavonoids and tannis, since these phytochemicals are reported to confer antibacterial activity [22, 23].

Following treatment with ginger oil and moringa oil individually and in combination with all the bacteria isolates from the fish samples, there was no significant difference effected (P>0.05) by the extracts, however, when compared with the controls there was significantly different (P<0.05) from Table 5.

Results suggests that the effect could be due to the presence of high amounts of flavonoids in the seed of *Moringa oleifera* and also the concentration of ginger used in this experiment inhibit from fatty acid (FFA) production. The FFA content in a product is an indication of the quality of the product.

The study therefore suggests that medicinal plant extracts such as ginger and Moringa should be used as alternative method as its action is indicative preventing emergence of resistant bacteria and improving the antimicrobial role as well as the quality of fish.

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