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RESEARCH ARTICLE

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The Potential of *Capsicum annum* Extracts to Prevent *Lactococcosis* in Tilapia

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Abstract

Capsaicin was extracted in-house from locally sourced chili pepper (*Capsicum annuum*) and tested for its bactericidal activity against *Lactococcus garvieae*. Serial dilutions of the extract were combined with *L. garvieae* and plated on Mueller-Hinton agar to determine the minimum bactericidal concentration (MBC). A total of 400 tilapia were divided into four groups (n = 100 per group) and injected with: (1) capsaicin alone, (2) *L. garvieae* and capsaicin, (3) *L. garvieae* alone, and (4) normal saline (negative control). Fish were monitored for seven days post-treatment, and the experiment was replicated three times. Protective efficacy was assessed based on survival rates and the absence of clinical disease. The MBC of capsaicin against *L. garvieae* was 0.1967 mg/mL. Survival was significantly higher in the group treated with both bacteria and capsaicin ($p < 0.0001$) compared to the bacteria-only group. These results indicate that chili pepper extract may confer protective effects against *L. garvieae* infection in tilapia.

KEYWORDS: *Tilapia*, *Lactococcus garvieae*, *Capsaicin*, *Capsicum annum*, *Antimicrobial Resistance, Ethnoveterinary medicine*

1.0 Introduction

Aquaculture plays a critical role in national economies by contributing to employment creation, income generation, and food production (Genschick et al., 2017; Tran et al., 2019). Over the past decade, the demand for farmed fish has increased significantly, largely due to the stagnation and eventual decline of fish harvests from capture fisheries. However, the rapid growth of the aquaculture sector has been accompanied by a rise in disease outbreaks, particularly in intensive production systems. In tilapia farming, several bacterial pathogens have been identified as major contributors to disease outbreaks, including *Aeromonas* *hydrophila*, *Streptococcus agalactiae*, *Streptococcus iniae*, and *Lactococcus garvieae* (Harikrishnan and Balasundaram, 2005; Amal and Zamri-Saad, 2011; Bwalya et al., 2020).

The most common treatment strategy for bacterial infections in aquaculture is the use of antibiotics (Meyburgh, Bragg and Boucher, 2017). Unfortunately, the widespread and often indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance (AMR), which has been well documented in several bacterial species affecting tilapia (Harikrishnan and Balasundaram, 2005; Meyburgh, Bragg and Boucher, 2017). Resistance development is influenced by national regulatory frameworks, which differ considerably across countries, affecting antibiotic accessibility and usage patterns.

The global rise of AMR in aquaculture pathogens has prompted increased research into sustainable, antibiotic-free disease control strategies. Among these, probiotics and ethnoveterinary medicines are emerging as viable alternatives. Probiotics, which include a broad range of beneficial Gram-negative and Gram-positive microorganisms such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Shewanella*, *Bacillus*, *Aeromonas*, *Vibrio*, *Enterobacter*, *Pseudomonas*, *Clostridium*, and *Saccharomyces* species, have shown promising results in disease prevention and health promotion in aquaculture systems (Cruz et al., 2012). Several studies have demonstrated the effectiveness of probiotics in mitigating bacterial infections in tilapia production (Hai, 2015).

Ethnoveterinary medicines—natural products of plant or animal origin used in traditional animal health care—offer low-cost and culturally accepted alternatives to conventional pharmaceuticals. These have been widely utilized in cattle, sheep, goats, and poultry production in regions such as Africa, South America, and Asia (Khan and Hanif, 2006; Confessor et al., 2009; Shen, Qian and Ren, 2010; Maroyi, 2012). One of the most notable ethnoveterinary plants is hot red pepper (*Capsicum annum*), a widely consumed spice from the *Solanaceae* family (Kobata et al., 1998). It has long been used both therapeutically and prophylactically in the treatment of various diseases in terrestrial animals (Akhtar et al., 2000; Shen, Qian and Ren, 2010; El-Deek et al., 2012).

In particular, *Capsicum annum* supplementation has been shown to significantly enhance body weight gain in broiler chickens when used alongside oxytetracycline (El-Deek et al., 2012). Capsaicin and other capsinoids found in red peppers exhibit broad-spectrum antimicrobial activity (Kobata et al., 1998). In addition to their antibacterial properties, *Capsicum* species are known for their immunostimulatory, anti-inflammatory, and digestive health benefits, as well as being rich in calcium (Omolo et al., 2014). Capsaicin, the primary active compound, is water-stable and has been shown in animal studies to be absorbed into the bloodstream (Diepvens, Westerterp and Westerterp-Plantenga, 2007).

This study aimed to evaluate the efficacy of *Capsicum annum* extracts in preventing *L. garvieae* infection in three-spot tilapia (*Oreochromis andersonii*) as a potential natural alternative to antibiotics in aquaculture.

2.0 Materials and Methods

2.1 Study design and ethical approval

This was a controlled laboratory-based experimental study conducted to evaluate the antibacterial efficacy of *Capsicum annum* extract (capsaicin) against *L. garvieae* infection in three-spot tilapia. The study adhered to the ethical guidelines of the National Health Research Ethics Committee of Zambia and received approval from the Excellence in Research Ethics and Science (ERES) Converge (Protocol Number: 2019-Aug-024). Fish handling and sampling were performed with minimal stress, and euthanasia was conducted upon first signs of clinical disease (lethargy, disorientation, and erratic swimming) through a blow to the head followed by cervical dislocation.

2.2 Study area and experimental fish

The study was carried out at the Wet Laboratory, School of Veterinary Medicine, University of Zambia. A total of 410 healthy three-spot tilapia, with an average weight of 100 ± 10 g, were sourced from Kalimba Crocodile and Fish Farm, located in Lusaka District. The farm had no known history of disease outbreaks. The fish were transported in oxygenated bags by road and acclimatized in 500 L flow-through tanks with dechlorinated water for 10 days. Tanks were aerated using stone bubblers, and water quality was monitored daily (temperature: 20 ± 2°C, dissolved oxygen: 7.9 ± 2 mg/L, pH: 7 ± 0.2). Fish were fed commercial pellets at 3% body weight daily.

2.3 Preparation of *Capsicum annum* Extract (Capsaicin)

Fresh chili peppers (*Capsicum annum*, 3 kg) were purchased from a local supermarket in Lusaka. No differentiation was made by batch or origin. At the University of Zambia, seeds were removed manually, and the remaining tissue was sun-dried for five days. The dried material (0.3 kg) was blended into a fine powder. Capsaicinoids were extracted following the method by Kurian and Starks (2002). Briefly, 50 mL methanol was added to the powder in a blender and homogenized for 5 minutes. The slurry was filtered using double Whatman GF/A glass fiber filters, and residual pulp was rinsed with additional methanol. The final volume of the filtrate was adjusted to 100 mL with methanol. This extract was submitted to the Zambia Bureau of Standards (ZABS) for High-Performance Liquid Chromatography (HPLC) analysis to quantify capsaicin content, according to Usman et al. (2014).

2.4 Determination of Metals Composition Growth Inhibition and Minimum Bactericidal Concentration (MBC) Assay

The capsaicin extract was serially diluted (1:10) into five concentrations. *L. garvieae* cultures were grown on Mueller-Hinton agar and standardized to 0.5 McFarland turbidity (Densimat, Biomerieux). Equal volumes (0.5 mL each) of the bacterial suspension and each capsaicin dilution were mixed and inoculated onto Mueller-Hinton agar plates using the spread plate technique. Plates were incubated at 25°C and examined daily for five days. The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of extract showing no visible bacterial growth. All tests were performed in triplicate.

2.5 Challenge Experiment

Ten fish were euthanized and tested prior to the experiment to confirm the absence of bacterial infections. The remaining 400 fish were randomly assigned to four experimental groups, with 100 fish per group. Group T1 (Control) received intraperitoneal injections of 0.1 mL sterile saline. Group T2 (Bacteria Only) was injected with *L. garvieae* suspension at a concentration of 9.6×10^5 CFU per fish. Group T3 (Bacteria + Capsaicin) received both *L. garvieae* at the same concentration and capsaicin at its minimum bactericidal concentration (MBC). Group T4 (Capsaicin Only) was injected with capsaicin at MBC. Each group was housed separately in 500-liter tanks (T1–T4) under identical environmental conditions. All fish were observed for seven days following injection for clinical signs of disease and mortality.

2.5 Data Collection and Management

Data collection tools included daily observation sheets for recording clinical signs and mortalities. Bacterial growth (colony counts) and capsaicin concentrations were recorded in laboratory notebooks and digital spreadsheets. Each sample and test condition was labeled and tracked throughout the study.

2.6 Data Analysis

Survival rates were calculated for each treatment group. Associations between treatment and survival outcomes were analyzed using Fisher's exact test and Chi-square test. A significance level of $p < 0.05$ was used. All statistical analyses were conducted using GraphPad Prism version 8.0 (www.graphpad.com).

3.0 RESULTS

Capsaicin concentrations in the extract and serial dilutions are presented in Table 1. The stock solution had a concentration of 1.967 mg/ml, prepared from an initial extract with 19.67 mg/g capsaicin content. Serial dilutions resulted in concentrations of 0.1967 mg/ml (1:10), 0.01967 mg/ml (1:100), 0.001967 mg/ml (1:1,000), and 0.0001967 mg/ml (1:10,000).

Table 1: Concentration of capsaicin (mg/ml) in the dilutions of the *Capsicum annum* extract

	Initial extract (mg/g)	Stock Solution (100ml)-mg/ml	Concentration in liquid (mg/ml) after serial dilutions			
			$1:10^1$ dilution	$1:10^2$ dilution	$1:10^3$ dilution	10^4 dilution
Capsaicin Concentration	19.67	1.967	0.1967	0.01967	0.001967	0.0001967

The antibacterial effect of *Capsicum annum* extract against *L. garvieae* was concentration-dependent, as shown in Table 2. Complete inhibition of bacterial growth was observed at capsaicin concentrations of 0.1967 mg/ml and 1.967 mg/ml, with zero colony-forming units (CFU) recorded on all five days of incubation. This indicates that 0.1967 mg/ml is the minimum bactericidal concentration (MBC) of the extract, as it was the lowest concentration at which no bacterial growth was observed throughout the study period.

At lower concentrations, partial inhibition was observed. The 0.01967 mg/ml treatment initially suppressed growth, but bacterial colonies appeared by Day 2 and increased progressively to 142 CFU/0.1 ml by Day 5, indicating it was below the MBC threshold. The 0.001967 mg/ml treatment showed minimal inhibitory effect, with bacterial counts increasing steadily from 25 CFU on Day 1 to 120 CFU by Day 3, plateauing thereafter.

Table 2: Colony forming unit of Lactococcus garvieae per 0.1ml with different concentrations of capsaicin

Day of incubation	Treatment 1: Capsaicin (0.001967mg/ml)	Treatment 2 Capsaicin (0.01967mg/ml)	Treatment 3 Capsaicin (0.1967mg/ml)	Treatment 4 Capsaicin (1.967mg/ml)
Day 1	25	0	0	0
Day 2	90	12	0	0
Day 3	120	50	0	0
Day 4	120	142	0	0
Day 5	120	142	0	0

Survivability trends of fish in the four treatment groups over six days post-treatment are illustrated in Figure 1. The *L. garvieae* + capsaicin group exhibited a survival rate of 80%, while the control group (normal saline) maintained the highest survival at 95%. In contrast, the *L. garvieae*-only group showed a sharp and continuous decline in survivability, with 0% survival by Day 5, indicating total mortality. The capsaicin-only group showed a delayed decline, with 20% mortality observed on Day 4, resulting in a final survival rate of 80% by Day 6.
Mortality in the *L. garvieae* + capsaicin group occurred early—20% of fish died by Day 2, after which survival stabilized. A statistically significant difference in fish survival was observed among the treatment groups ($p < 0.0001$).

4.0 DISCUSSION

This study demonstrated that *Capsicum annum* extract possesses antibacterial activity against *L. garvieae*, a significant pathogen in aquaculture. The capsaicin concentration in the locally sourced chili was determined to be 19.67 mg/g, and a concentration of 0.1967 mg/ml was identified as the minimum bactericidal concentration (MBC) effective in inhibiting the growth of *L. garvieae* in vitro. These findings are consistent with previous studies, which have reported capsaicin MIC values ranging from 0.1 to 0.2 mg/ml (Fieira et al., 2013; Marini et al., 2015). Marini et al. (2015) found that both erythromycin-resistant and susceptible bacterial strains had MICs clustered around 0.128 mg/ml, while the highest MIC observed was 0.512 mg/ml. Similarly, Careaga et al. (2003) observed variable inhibitory effects of capsaicin against *Salmonella typhimurium* in raw meat, and additional studies have documented inhibitory effects of *Capsicum* extracts on *S. typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* (Koffi-Nevry et al., 2012).

In the in vivo challenge, administration of *Capsicum annum* extract significantly improved fish survival. Eighty percent of tilapia injected with both *L. garvieae* and capsaicin survived the trial period, whereas the group infected with *L. garvieae* alone had 100% mortality ($p < 0.0001$). This suggests that capsaicin not only has direct antimicrobial activity but may also interfere with the pathogenesis of *L. garvieae* in fish. Similar effects have been reported in terrestrial animals; for example, Hanif et al. (2012) found that *Capsicum annum* effectively controlled Newcastle Disease in broilers. Additionally, its historical use in ethnoveterinary medicine highlights its potential for integration into livestock health management (Cichewicz and Thorpe, 1996; Guèye, 2002).
The findings of this study have important policy implications for antimicrobial use in aquaculture. With growing concerns over AMR driven by the overuse of antibiotics in fish farming, the use of ethnoveterinary products like *Capsicum annum* could serve as a sustainable,

low-cost alternative to conventional antibiotics. Given that aquaculture systems can act as reservoirs for resistant bacteria and facilitate horizontal gene transfer among aquatic organisms (Watts et al., 2017), incorporating plant-based antimicrobials into disease control protocols can help reduce the sector's contribution to AMR. These findings support the formulation of national guidelines or standards that promote the evaluation, approval, and responsible integration of natural antimicrobials in aquaculture health management programs.

While the study provides promising evidence for the efficacy of capsaicin, it has several limitations. First, the study focused on a single pathogen, *L. garvieae*, and results may not be generalizable to other common bacterial pathogens in aquaculture. Second, capsaicin concentration and activity may vary depending on chili variety, growing conditions, and extraction method—factors not standardized in this study. Furthermore, although survival data were collected, no histopathological or immunological assays were conducted to better understand capsaicin's mechanism of action in fish.

This study opens several avenues for further research. Future studies should explore the efficacy of *Capsicum annum* extract against a broader range of fish pathogens and in different aquaculture species. Dose optimization studies are also needed to assess long-term safety and determine whether capsaicin affects growth performance, immunity, or reproduction. Investigating the mode of action of capsaicin in aquatic organisms—whether through direct bacterial inhibition or immunomodulatory effects—would provide valuable insights. Additionally, developing standardized formulations (e.g., feed additives or water treatments) and testing under field conditions will be essential for commercial application.

Conclusion

This study demonstrated that *Capsicum annum* extract, specifically capsaicin at a concentration of 0.1967 mg/ml, exhibits effective antibacterial activity against *L. garvieae* in vitro and significantly improves survivability in infected tilapia. These findings support the potential of *Capsicum annum* as a natural, cost-effective alternative to antibiotics in aquaculture, contributing to efforts to reduce antimicrobial resistance.

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Conflict of interest

The authors declare no conflict of interest.

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APPENDICES: FIGURES

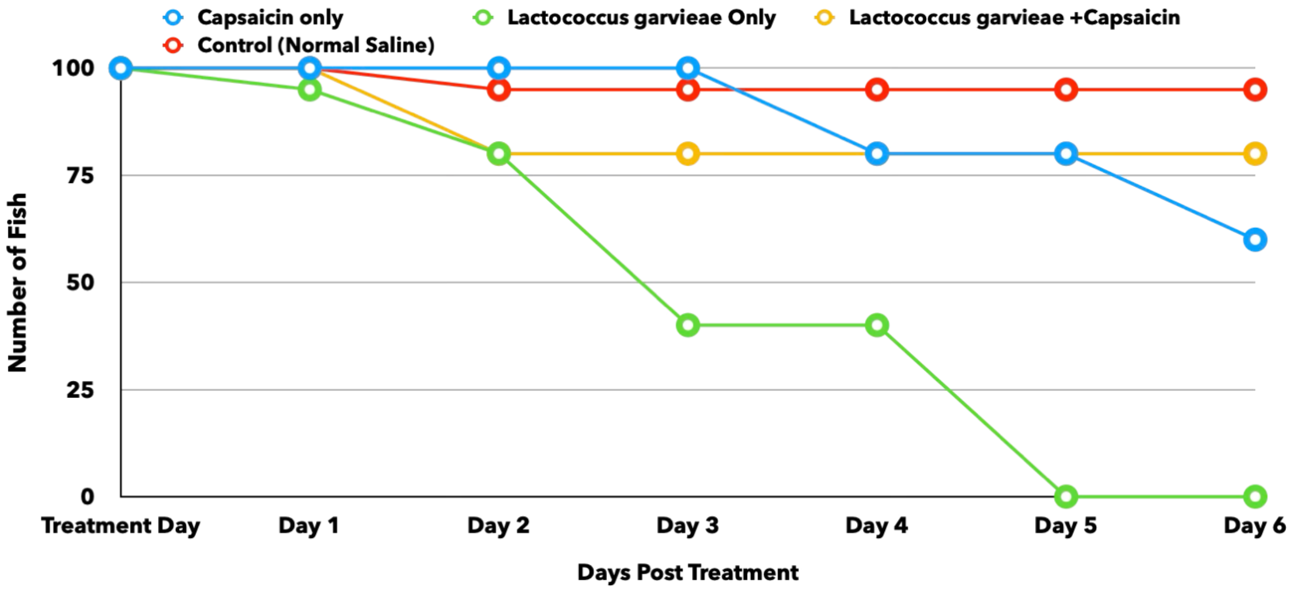

Figure 1: survivability of the fish in the different treatment groups over period of 6 days post treatment