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Research Article

Effect of *Pseudomonas aeruginosa* infection on the survival of Nile tilapia (*Oreochromis niloticus*) fed with aloe vera (*Aloe barbadensis*)

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ABSTRACT. Medicinal plants have been increasingly used in aquaculture nutrition as natural alternatives to antibiotics to control bacterial diseases, including those caused by *Pseudomonas aeruginosa*. *Aloe barbadensis* Miller is recognized as a medicinal plant rich in bioactive compounds that can improve survival and enhance resistance to pathogenic agents. This study evaluated the survival of *Oreochromis niloticus* fed diets supplemented with *A. barbadensis* and challenged with *P. aeruginosa* via immersion and intraperitoneal injection, without considering immune parameters. Three bioassays were conducted with Nile tilapia juveniles: bioassay 1 (2.86 ± 0.26 g), bioassays 2 and 3 (3.25 ± 0.30 g). In bioassay 1, infection was performed by immersion, and in bioassay 2 by intraperitoneal injection; in both cases, diets containing *A. barbadensis* (0, 0.5, 1.5, and 2.5% kg^{-1} of feed) were evaluated. In bioassay 3, only the 1.5% concentration was tested by intraperitoneal injection, and a *post-mortem* examination was performed. The results showed that in bioassay 1, the lowest survival rate (80%) was observed in TIV (2.5% *A. barbadensis*), which differed significantly ($P < 0.05$) from the control TI (100%). In bioassay 2, the highest survival rate (90%) was observed in TI, with significant differences ($P < 0.05$) compared to TII and TV. In bioassay 3, TII without *A. barbadensis* showed the lowest survival rate (52.38%), which differed significantly from TI and TIII ($P < 0.05$). Therefore, this study enabled a controlled evaluation of the effect of *A. barbadensis* on the survival of *O. niloticus* by reducing mortality during *P. aeruginosa* infection.

Keywords: *Aloe barbadensis*; *Oreochromis niloticus*; *Pseudomonas aeruginosa*; survival rate; feed additive

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INTRODUCTION

The culture of Nile tilapia (*Oreochromis niloticus*) faces significant challenges related to aquatic animal health and biosafety, owing to the acquisition of infections during production cycles and the risks these pose for the spread of infectious bacterial diseases and their consequent impacts on production (Bondad-Reantaso et al. 2023, Abaho et al. 2025). The principal bacterial families associated with disease in the culture of *O. niloticus* are Pseudomonadaceae, Aeromonadaceae, Vibrionaceae, Streptococcaceae, and Staphylococcaceae (Ali et al. 2021). Their control has largely relied on excessive use of antibiotics and biocides, thereby contributing to the emergence of resistant pathogenic strains (Hernández et al. 2021, Chavan & Vashishth 2025).

Bacteria of the genus *Pseudomonas* are among the most prevalent pathogens in fish culture. They are associated with ulcerative diseases, including ulcerative syndrome, thereby posing a serious threat to aquaculture health and a potential concern for human consumers (Alzahrani et al. 2023, Qadiri 2023). These Gram-negative bacteria act as opportunistic pathogens, and although *P. aeruginosa* is part of the normal microbiota of fish, it is frequently reported as an etiological agent of septicemia in aquatic organisms (Algammal et al. 2020, El-Tarabili et al. 2023, Mohamed et al. 2023). Under conditions of stress or immunosuppression, its proliferation rises, thereby increasing the risk of systemic infections and production losses (Osman et al. 2021, Suresh et al. 2023). Consequently, its impact on aquaculture is substantial, with estimated economic losses of US$ 6 billion worldwide and approximately 15% of total production losses (Assefa & Abunna 2018, Amillano-Cisneros et al. 2025).

In response to the challenge posed by potential bacterial diseases, there is a need to develop new control strategies in aquaculture, including the use of medicinal plants, effective and capable of enhancing growth and survival, economically viable, environmentally sustainable, and characterized by low toxicity (Maulu et al. 2021, Liao et al. 2022, Tadese et al. 2022). These beneficial effects are attributed to bioactive compounds that exhibit a wide range of properties, including antistress, antioxidant, anti-inflammatory, antimicrobial, antifungal, antiparasitic, and antiviral activities (Nair et al. 2021, Wei et al. 2022, Dhama et al. 2023).

Several medicinal plants have been reported to act as immunostimulants in juvenile *O. niloticus*, owing to their antimicrobial and antiparasitic properties against bacterial infections. Dietary supplementation with Mojave yucca (*Yucca schidigera*) improved growth, survival, and disease resistance (El-Keredy & Naena 2020), whereas supplementation with parsley (*Petroselinum sativum* Hoffm.) enhanced both humoral and cellular immunity in Nile tilapia challenged with *P. aeruginosa* (El-Houseiny et al. 2022). In addition, oregano (*Origanum vulgare*) exhibited hepatoprotective and nephroprotective effects, further highlighting its antimicrobial potential (Rehan et al. 2024).

From this perspective, *A. barbadensis* has attracted growing attention as a functional additive in aquaculture because, in addition to its anti-inflammatory and immunomodulatory properties, it has shown consistent antimicrobial activity against several bacterial pathogens of veterinary and aquacultural relevance (Kumar et al. 2019). Also, *in vitro* studies have demonstrated that the antibacterial effect of *A. barbadensis* is concentration-dependent: a 20% extract inhibited only *Escherichia coli*, whereas extracts at 40 and 80% exhibited broader inhibitory activity against *P. aeruginosa*, *Staphylococcus aureus*, and *E. coli* (Mukundan & Thangavelu 2025). Similarly, other recent studies have reported that *A. barbadensis* showed greater inhibitory activity against *P. aeruginosa*, which is particularly relevant given the opportunistic and pathogenic role of this bacterium in cultured fish (Abdelgader et al. 2024, Fadhil et al. 2024). In parallel, *A. barbadensis* has also shown protective effects in vivo, as its dietary supplementation reduced mortality in red tilapia challenged with *Streptococcus agalactiae*, improved survival in Nile tilapia following *Aeromonas hydrophila* infection when combined with propolis, and decreased mortality by up to 20% in Nile tilapia challenged with *Lactococcus garvieae* (Sharifah et al. 2016, Dotta et al. 2018, Chilukutu et al. 2025).

Similar beneficial responses have been documented in other cultured fish species, including *Acipenser baerii*, *Labeo rohita*, and *Clarias gariepinus*, in which supplementation with *A. barbadensis* improved growth, survival, physiological performance, innate immune response, and resistance to bacterial challenge (Haghighi et al. 2016, Palaniyappan et al. 2023, Nwanna & Ikuesan 2025). In addition, *A. barbadensis* has been reported as a natural antimicrobial agent against *Streptococcus pyogenes*, *S. iniae*, *A. hydrophila*, and *S. agalactiae*, thereby supporting its broad-spectrum antibacterial potential and its possible application as a natural alternative to conventional chemotherapeutics in aquaculture (Ferro et al. 2003, Gabriel et al. 2015, Manaf et al. 2016, Zanuzzo et al.

2017, Mbokane & Moyo 2022). Therefore, the present study hypothesized that the inclusion of *A. barbadensis* Miller (0.5-2.5% kg^{-1} feed) would affect survival following challenge with *P. aeruginosa*.

MATERIALS AND METHODS

Ethical considerations

Juvenile *O. niloticus* were handled and sacrificed following the Mexican Official Standard NOM-062-Z00-1999 for the welfare, care, and appropriate use of laboratory animals and experimental testing.

Experimental animals

A total of 120 juvenile *O. niloticus* with an initial mean weight of 2.86 ± 0.26 g were used in bioassay 1, whereas 330 fish with a mean weight of 3.25 ± 0.30 g were used in bioassays 2 and 3. Fish were obtained from the Unidad Nayarit of the Centro de Investigaciones Biológicas del Noroeste S.C. (UNCIBNOR, Nayarit, Mexico). A prophylactic treatment was applied for 3 min in 100 L of filtered water (20 µm) with 3% NaCl. Fish were then acclimated for five days in a 200 L tank under controlled conditions of temperature 25-30°C (Leonard & Skov 2022), dissolved oxygen (DO) 5-7 mg L^{-1} (Abd El-Hack et al. 2022), and pH 6.5-9 (Kelany et al. 2024). They were fed three times daily (08:00, 12:00, and 17:00 h) with commercial tilapia feed (Silver Cup®, 32% protein, 5% fat) at 3% of total biomass. Uneaten feed and waste were siphoned daily before feeding.

Acclimation to experimental conditions

After acclimation, the fish were randomly assigned to five groups of 30 ind each, with each group divided into three replicates of 10 fish. They were housed in experimental aquaria with a capacity of 6 L containing 4 L of filtered water (20 µm) under continuous aeration. Environmental parameters were maintained as described above. Over 15 days, fish were fed three times daily (08:00, 12:00, and 17:00 h) with diets supplemented with *A. barbadensis* at concentrations of 0% (control), 0.5, 1.5, and 2.5%. Feeding was adjusted weekly to 3% of total biomass. Uneaten feed and feces were siphoned daily before feeding.

Preparation of experimental diets containing *A. barbadensis*

A. barbadensis plants were collected from home gardens in Tepic, Nayarit, Mexico. Leaves were disinfected with 1% NaClO, oven-dried at 45°C for 48 h, and ground to a fine powder (Hamilton Beach®). Commercial feed was pulverized and mixed with *A. barbadensis* powder at concentrations of 0.5, 1.5, and 2.5% kg^{-1} feed, with 0.5% gelatin and 300 mL of distilled water, to form a homogeneous paste. The control diet contained only commercial feed, 0.5% gelatin, and 300 mL of distilled water. All mixtures were pelletized using an electric meat grinder (Ship to Shore®) and air-dried at 28°C for 48 h. Pellets were stored at 4°C until use.

Preparation of *P. aeruginosa* bacterial inoculum

The *P. aeruginosa* strain used in this study had been previously isolated and characterized by Trejo-Flores et al. (*in press*). A bacterial culture was initiated from stock in tryptic soy broth (TSB) and incubated at 30°C for 24 h until reaching an optical density (OD_{600}) of 0.5 (log phase). The culture was centrifuged (50 mL aliquots) at 3,000 *g* for 20 min, and the pellet was resuspended in 1 mL of phosphate-buffered saline (PBS, pH 7.4) as described by Fierro-Coronado et al. (2019). The suspension was adjusted to an OD_{600} of 1.0 using a spectrophotometer. The CFU mL^{-1} concentration was estimated by plate counting on tryptic soy agar (TSA) after 24 h of incubation at 30°C using the serial dilution method.

Experimental design

Determination of the median lethal concentration (LC_{50}) of *P. aeruginosa*

A 6 days bioassay was conducted to determine the LC_{50} of *P. aeruginosa* via immersion, following the method of Fierro-Coronado et al. (2019). Using juvenile *O. niloticus* (2.86 ± 0.26 g), five treatments were evaluated in a completely randomized design with three replicates per treatment and 10 fish per tank, as follows: TI) control (no bacteria); TII) 1×10^4 CFU mL^{-1}; TIII) 1×10^5 CFU mL^{-1}; TIV) 1×10^6 CFU mL^{-1}; TV) 1×10^7 CFU mL^{-1}.

A second LC_{50} bioassay (6 days) was conducted by intraperitoneal injection on the left side of the lateral line of each fish, administering a single 3 µL dose of bacterial inoculum, as described by Rajme-Manzur et al. (2023). Five treatments were evaluated and randomly assigned. (triplicates, 10 fish per tank): TI) control (no bacteria); TII) 1×10^4 CFU mL^{-1}; TIII) 1×10^5 CFU mL^{-1}; TIV) 1×10^6 CFU mL^{-1}; TV) 1×10^7 CFU mL^{-1}. Fish were fed as previously described, and mortality was recorded three times daily. Water temperature was maintained at 28-30°C to ensure optimal *P. aeruginosa* development. LC_{50} values were calculated by Probit analysis (Finney 1971).

Bioassay 1. Survival of *O. niloticus* fed *A. barbadensis* and challenged with *P. aeruginosa* by immersion.

A 15-day bioassay was conducted using juvenile *O. niloticus* (2.86 ± 0.26 g) stocked in 6 L tanks containing 4 L of water (10 fish per tank). Fish were challenged by immersion with the LC_{50} concentration (5×10^6 CFU mL^{-1}), as described by Fierro-Coronado et al. (2019). Diets supplemented with *A. barbadensis* were administered for 15 days before infection to allow its bioactive compounds to exert antimicrobial and anti-inflammatory effects, as suggested by Hardi et al. (2018). The experimental design consisted of four treatments, each randomly assigned in triplicate, as follows: TI) control (commercial feed + 0.5% NaCl saline); TII) 0.5% *A. barbadensis* + feed + *P. aeruginosa* LC_{50}; TIII) 1.5% *A. barbadensis* + feed + *P. aeruginosa* LC_{50}; TIV) 2.5% *A. barbadensis* + feed + *P. aeruginosa* LC_{50}.

Fish were fed at 3% of biomass three times daily (08:00, 12:00, and 17:00 h). The experimental system was maintained under controlled conditions, with temperature ranging from 25 to 30°C (Leonard & Skov 2022), DO ranging from 5 to 7 mg L^{-1} (Abd El-Hack et al. 2022), and pH ranging from 6.5 to 9.0 (Kelany et al. 2024). Mortality was recorded daily.

Bioassay 2. Survival of *O. niloticus* fed *A. barbadensis* and challenged with *P. aeruginosa* by intraperitoneal injection

Following bioassay 1, a second 15-day bioassay was conducted with *O. niloticus* juveniles (3.25 ± 0.30 g). After 15 days of dietary treatment, fish were injected intraperitoneally with *P. aeruginosa* at the LC_{50} concentration (3×10^6 CFU mL^{-1}). The bioassay consisted of five randomly assigned treatments (triplicates, 10 fish per tank): TI) control (feed + sterile 0.9% NaCl solution); TII) feed + *P. aeruginosa*; TIII) 0.5% *A. barbadensis* + feed + *P. aeruginosa*; TIV) 1.5% *A. barbadensis* + feed + *P. aeruginosa*; TV) 2.5% *A. barbadensis* + feed + *P. aeruginosa*.

Fish were fed at 3% biomass three times daily (08:00, 12:00, and 17:00 h). Tanks were siphoned every third day. It was maintained under controlled temperature conditions of 25 to 30°C (Leonard & Skov 2022), a DO of 5 to 7 mg L^{-1} (Abd El-Hack et al. 2022), and a pH of 6.5 to 9 (Kelany et al. 2024), and mortality was recorded daily.

Bioassay 3. Confirmation of the effect of *A. barbadensis* against *P. aeruginosa* inoculated by injection

The third bioassay (5 days) was conducted simultaneously with bioassay 2, using a 1.5% *A. barbadensis* concentration to confirm its effect against the *P. aeruginosa* challenge. Nile tilapia juveniles (3.25 ± 0.30 g) were maintained in 6 L aquaria containing 4 L of water, with 20 fish per tank. Fish were infected following the methodology of Rajme-Manzur et al. (2023) via intraperitoneal injection applied along the lateral line of each tilapia. Each fish received 3 µL of *P. aeruginosa* bacterial inoculum ($LC_{50} = 3\times10^6$ CFU mL^{-1}). Three treatments were randomly assigned, each in triplicate: TI) control with commercial feed (CF) without *P. aeruginosa* + 1.5% *A. barbadensis* + injection of sterile 0.9% NaCl saline solution; TII) CF + *P. aeruginosa* ($LC_{50} = 3\times10^6$ CFU mL^{-1}) and TIII) CF + 1.5% *A. barbadensis* + *P. aeruginosa* ($LC_{50} = 3\times10^6$ CFU mL^{-1}). Fish were fed the experimental diets at 3% of their biomass, three times daily (08:00, 12:00, and 17:00 h). Aquaria were siphoned on the third day of the bioassay. It was maintained under controlled temperature conditions of 25 to 30°C (Leonard & Skov 2022), DO of 5 to 7 mg L^{-1} (Abd El-Hack et al. 2022), and pH of 6.5 to 9 (Kelany et al. 2024), with mortality recorded daily. Finally, *post-mortem* examination was performed on three diseased fish per treatment to identify external and internal clinical signs.

Isolation and molecular identification of *P. aeruginosa* from infected tissue

To confirm the genetic identity of the *Pseudomonas* strain, samples were cultured on TSA plates and incubated for 24 h. After incubation, they were sent to LabSerGen (Laboratorio de Servicios Genómicos, UGA-LANGEBIO, Irapuato, Guanajuato, Mexico) for molecular identification. Sequencing of the 16S rRNA gene was performed using primers p16SA (F and R), targeting regions V1-V4, and p16SB (F and R), targeting regions V5-V9. Sequence analysis was conducted using the GenBank database from the National Center for Biotechnology Information (NCBI) with the Basic Local Alignment Search Tool (BLAST) program, and the results were confirmed against the strain data from Trejo-Flores et al. (*in press*).

Statistical analysis

Survival data from each bioassay were arcsine-transformed as described by Daniel (1997). Data normality was assessed using the Shapiro-Wilk test, whereas homogeneity of variances was evaluated by Bartlett's and Levene's tests. A one-way analysis of variance (ANOVA) was performed for each bioassay, and statistically significant differences among experimental diets were identified using Tukey's HSD test. All statistical analyses were conducted at a significance level of $P < 0.05$ using STATISTICA 7® software.

Survival curve analysis was performed using the Kaplan-Meier method in SPSS version 20, and relative percent survival (RPS) was calculated according to Amend (1981) using the following formula:

$$\text{RPS} = 1 - \left(\frac{\%\ \text{mortality in the treated group}}{\%\ \text{mortality in the control group}}\right) \times 100$$

RESULTS

Water quality parameters

Water quality parameters were measured in all experimental aquaria. Mean water temperature, DO, and pH values remained within the recommended ranges for Nile tilapia culture. In bioassay 1, temperature was 28 ± 0.6°C, DO was 5.26 ± 0.1 mg L^{-1}, and pH was 7.28 ± 0.2. In bioassay 2, temperature was 28°C, DO was 5.1 mg L^{-1}, and pH was 7.0. In bioassay 3, temperature was maintained at 28°C, DO was 5.17 mg L^{-1}, and pH was 7.08.

Bioassay 1. Survival of *O. niloticus* fed with *A. barbadensis* and challenged by immersion with *P. aeruginosa*

No mortality was recorded in treatment TI (uninfected control); therefore, RPS was not calculated. In contrast, the first mortality in TIV was recorded on day 4, and survival reached 80%, showing significant differences ($P = 0.004$) relative to the control treatment (100%). By comparison, in treatments TII (93.73%) and TIII (96.67%), mortality was first observed after 7 days post-infection; accordingly, no significant differences were detected between these treatments ($P > 0.05$) (Figs. 1-2).

Bioassay 2. Survival of *O. niloticus* fed with *A. barbadensis* and challenged by injection with *P. aeruginosa*

The results of bioassay 2 are presented in Figures 3-4. Following intraperitoneal infection with *P. aeruginosa*, the first mortality was recorded on day 1 in treatments TII, TIII, TIV, and TV, whereas in TI it was first observed on day 7. Accordingly, TI showed the highest survival rate (90%), with significant differences ($P < 0.05$) relative to TII (23.33%), TIII (23.33%), TIV (53.33%), and TV (43.33%). As shown in Figure 3, the uninfected control treatment exhibited the highest survival, significantly exceeding that of all infected groups. The RPS values did not exceed the 60% threshold proposed by Amend (1981) for effective protection, with RPS values of 39.13% for TIV and 29.06% for TV. Nevertheless, the diet supplemented with 1.5% *A. barbadensis* yielded the highest survival among the infected treatments. It differed significantly ($P < 0.05$) from the infected control, suggesting a protective effect of the plant extract against bacterial

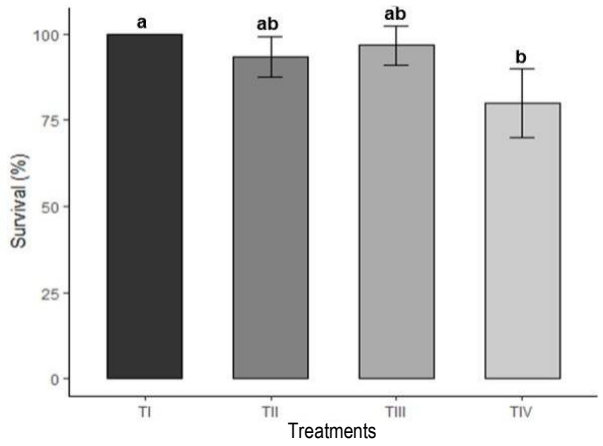

Figure 1. Survival (mean ± standard deviation) of *Oreochromis niloticus* fed with powdered *Aloe barbadensis* and challenged by immersion with *Pseudomonas aeruginosa*. Treatments: I) control with commercial feed (CF) without *Pseudomonas* + 0.5% NaCl saline solution, II) 0.5% *A. barbadensis* + CF + *Pseudomonas* LC_{50}, III) 1.5% *A. barbadensis* + CF + *Pseudomonas* LC_{50}, and IV) 2.5% *A. barbadensis* + CF + *Pseudomonas* LC_{50}. Different lowercase superscripts indicate statistically significant differences ($P < 0.05$).

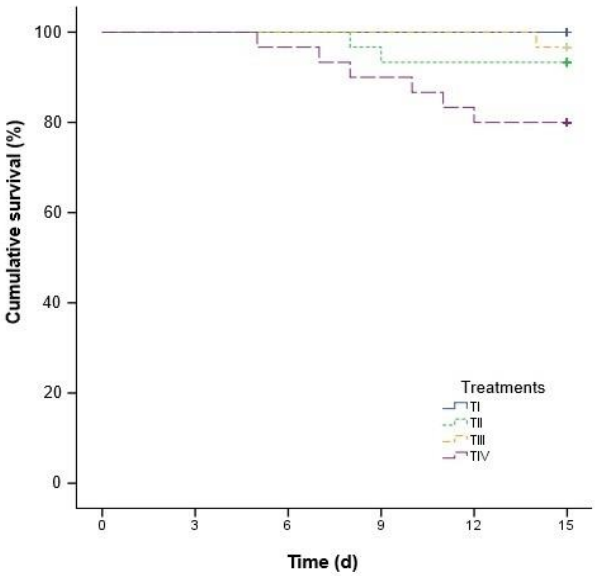

Figure 2. Mean survival percentage of treatments fed *A. barbadensis* compared with the control group (uninfected) during the 15 days following immersion challenge with *P. aeruginosa*. Statistical analysis of cumulative survival was performed using the Kaplan-Meier survival curve (n = 30 fish per treatment) ($P < 0.05$).

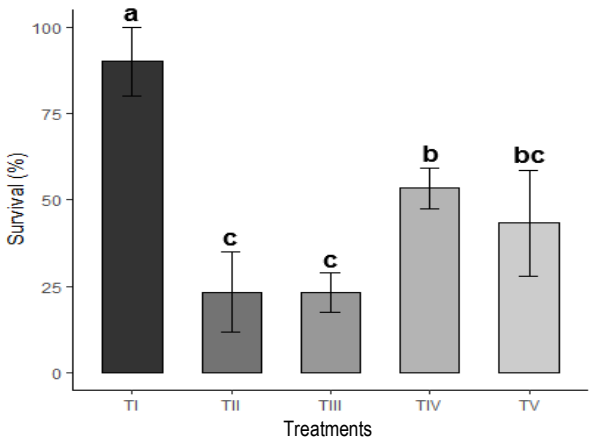

Figure 3. Survival (mean ± standard deviation) of *Oreochromis niloticus* fed diets containing powdered *Aloe barbadensis* and challenged by injection with *Pseudomonas aeruginosa*. The control group received commercial feed without *P. aeruginosa* and an intraperitoneal injection of sterile 0.9% NaCl solution, while the infected control group received only commercial feed and *P. aeruginosa*. The experimental treatments consisted of fish fed commercial feed supplemented with 0.5, 1.5, or 2.5% *A. barbadensis*, followed by challenge with *P. aeruginosa*. Different lowercase superscripts indicate statistically significant differences ($P < 0.05$).

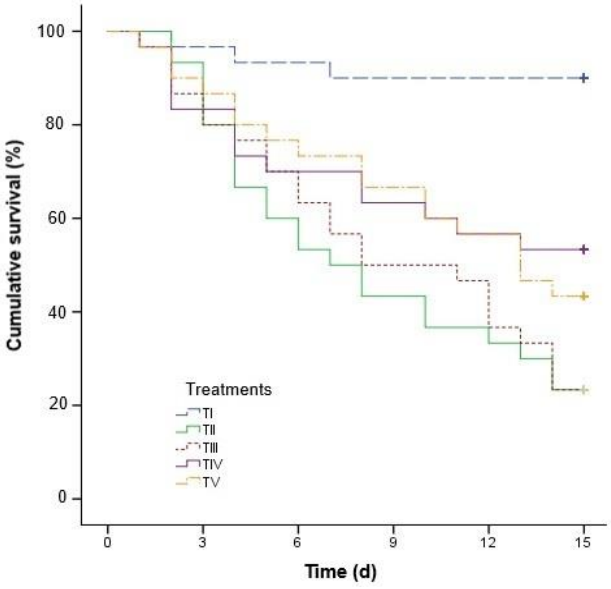

Figure 4. Mean survival percentage of treatments fed *A. barbadensis* compared with the control groups (uninfected and without *A. barbadensis*) during the 15 days following challenge by injection with *P. aeruginosa*. Statistical analysis of cumulative survival was performed using the Kaplan-Meier survival curve (n = 30 fish per treatment) ($P < 0.05$).

challenge. Within the groups fed *A. barbadensis*, the 1.5% inclusion level also showed significant differences ($P = 0.003$) relative to the 0.5% treatment.

Bioassay 3. Confirmation of the effect of *A. barbadensis* against *P. aeruginosa* by injection.

Survival and post-mortem examination

Figure 5 shows the highest survival in the control group (68.25%) and the lowest in the infected group without *A. barbadensis* (52.38%), with significant differences ($P = 0.005$). However, the control treatment did not differ significantly from the 1.5% *A. barbadensis* group (63.49%). These findings suggest a protective effect of *A. barbadensis* against *P. aeruginosa* infection ($LC_{50} = 3\times10^6$ CFU mL^{-1}). Survival increased by approximately 10% in fish fed a diet containing 1.5% *A. barbadensis* combined with commercial feed before bacterial challenge. Figure 6 shows that mortality began on day 1 and continued through day 5 of the evaluation period. The RPS calculated for treatment TIII was 23.33%. Fish exhibited petechial hemorrhages throughout the body, particularly on the head, at the base of the fins, and on the caudal peduncle. Additional clinical signs included scale loss, caudal fin erosion, skin darkening, dermal ulcerations, and exophthalmia (Fig. 7). The coloration and appearance of the internal organs of *O. niloticus* infected with *P. aeruginosa* were altered compared with those of the control group, showing evident damage to internal tissues, including pale liver and pancreas and a darkened gallbladder (Fig. 8).

Isolation and molecular identification of *P. aeruginosa* from infected tissue

At the end of the study, presumptive *Pseudomonas* colonies were isolated on cetrimide agar from the muscle tissue of fish from the confirmatory bioassay in the infected control and *A. barbadensis* -treated groups. BLAST homology analysis revealed that both the strain used for infection and the *Pseudomonas* isolates obtained from these treatments matched *P. aeruginosa* DSM 50071 (Accession No. NR_117678.1) with 97.62% sequence similarity.

DISCUSSION

Since ancient times, medicinal plants have been used for therapeutic purposes and disease prevention, gradually gaining importance in both human and veterinary medicine (Cáceda et al. 2023). In aquaculture, the incorporation of medicinal plants into fish diets has become a highly promising strategy to address the emergence of bacterial diseases, particu-

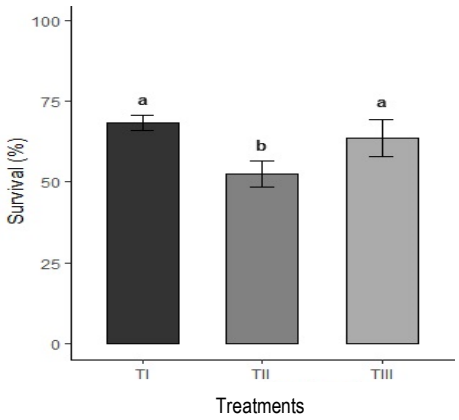

Figure 5. Bioassay confirming survival (mean ± standard deviation) of *Oreochromis niloticus* fed with the optimal concentration of powdered *Aloe barbadensis* and challenged by intraperitoneal injection with *Pseudomonas aeruginosa*. Treatments: I) control group fed with commercial feed (CF) without *P. aeruginosa* + 1.5% *A. barbadensis* + intraperitoneal injection of sterile 0.9% NaCl solution; II) CF + *P. aeruginosa* ($LC_{50} = 3\times10^6$ CFU mL^{-1}); and III) CF + 1.5% *A. barbadensis* + *P. aeruginosa* ($LC_{50} = 3\times10^6$ CFU mL^{-1}). Different lowercase superscripts indicate statistically significant differences ($P < 0.05$).

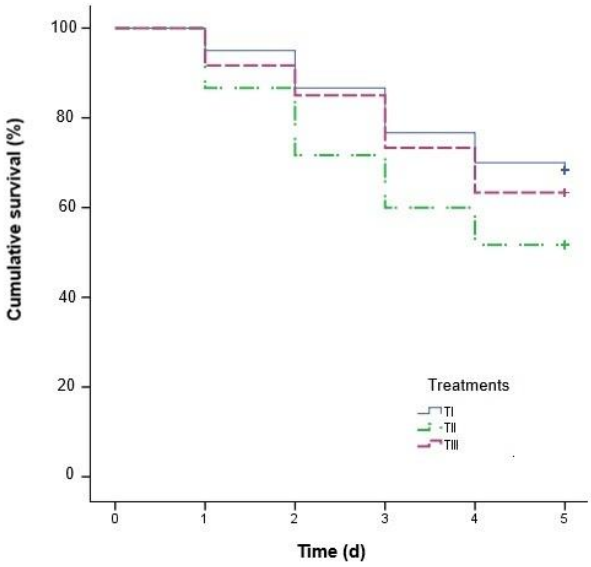

Figure 6. Mean survival percentage of treatments fed 1.5% powdered *A. barbadensis* compared with the control group (uninfected and without *A. barbadensis*) during the five days following challenge by injection with *P. aeruginosa*. Statistical analysis of cumulative survival was performed using the Kaplan-Meier survival curve ($P < 0.05$) (n = 60 fish per treatment).

larly those caused by *P. aeruginosa* (Usman et al. 2023), as these plants can serve as growth promoters, immunostimulants, sex-reversal agents, and antimicrobials, among other functional roles (Kuebutornye & Abarike 2020, Gabriel et al. 2022).

Several studies in different fish species have evaluated the antimicrobial activity of *A. barbadensis* and its main constituents, focusing primarily on bacteria such as *Streptococcus iniae* (Gabriel et al. 2015), *Aeromonas hydrophila* (Zanuzzo et al. 2017, Dotta et al. 2018, De Assis & Urbinati 2020, Srivastava et al. 2023), *Staphylococcus aureus*, and *P. aeruginosa* (Sánchez et al. 2020). However, no previous studies have addressed *Pseudomonas* infections in tilapia treated with *A. barbadensis*, underscoring the significance of the present study as a foundation for future research evaluating the antimicrobial effects of *A. barbadensis* in Nile tilapia and other aquatic organisms.

In this study, the clinical signs observed in Nile tilapia infected with *P. aeruginosa* were consistent with reports by El-Nagar (2010), Saad-El-Deen (2014), and Abd El-Tawab et al. (2019), including body hemorrhages, exophthalmia, scale detachment, skin darkening, and ulcerations. Internally, congestion and pallor of the liver and pancreas, along with darkening and enlargement of the gallbladder, were observed, indicative of septicemia caused by *P. aeruginosa*. Across the three bioassays conducted, fish fed *A. barbadensis* following bacterial challenge exhibited higher survival, particularly those receiving 1.5% supplementation per kilogram of feed, as further illustrated by the Kaplan-Meier survival curves. Treatment with *A. barbadensis* in Nile tilapia reduced mortality following challenge with *P. aeruginosa*, achieving RPS values of 23.33% in bioassay 1 and 39.13% in bioassay 2, indicating a moderate reduction in mortality relative to the positive control. This protective effect is consistent with the findings of El Araby & El-Arabey (2016), who reported 100% survival in Nile tilapia fed 5 and 10 g kg^{-1} of *Origanum vulgare* extract and challenged with *P. aeruginosa*.

In contrast, the 0 g kg^{-1} control group exhibited 93.3% survival. This effect may be attributed to the essential oil and extracts of *O. vulgare*, which contain bioactive compounds with antimicrobial, antioxidant, and other biological properties that may enhance Nile tilapia's resistance to disease. Likewise, Charo et al. (2023) reported that tilapia fed *A. barbadensis* Miller showed inhibition of bacterial growth in gill isolates at concentrations ranging from 125 to 250 mg mL^{-1}, specifically against *Pseudomonas mendocina* and *P. putida*.

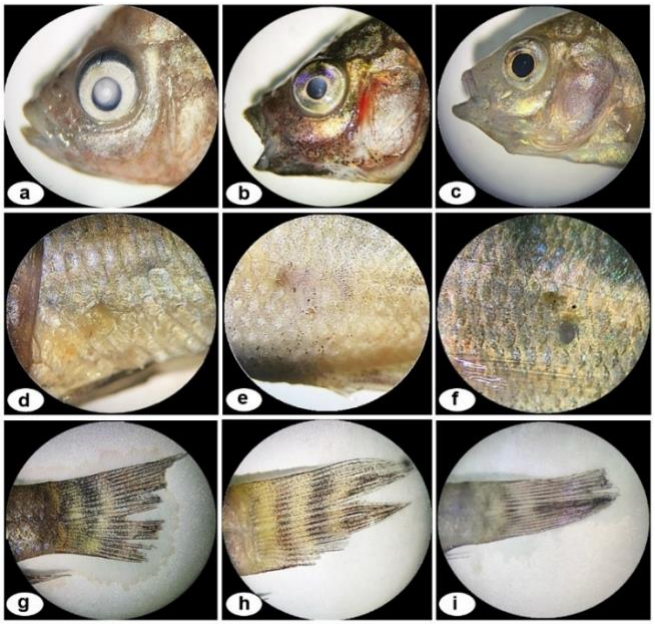

Figure 7. *Post-mortem* observation of clinical signs in *Oreochromis niloticus* fed 1.5% powdered *Aloe barbadensis* and challenged by intraperitoneal injection with *Pseudomonas aeruginosa*. a-c) Exophthalmia and petechial hemorrhages; d-f) skin desquamation and ulceration; and g-i) caudal fin necrosis (n = 3 fish per treatment).

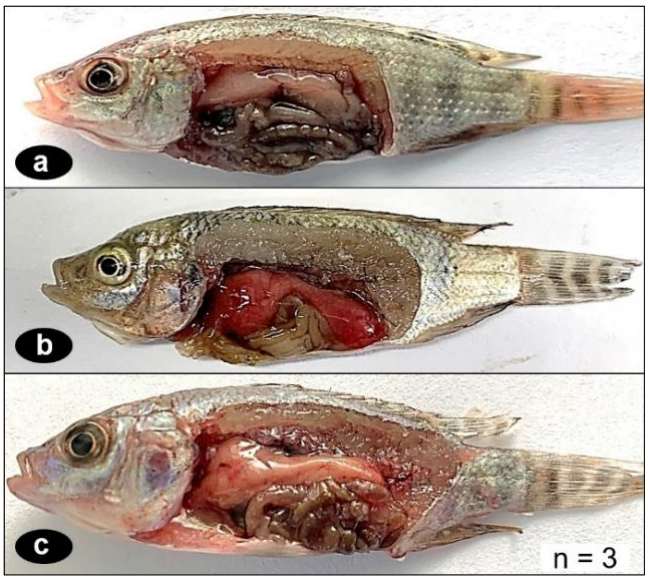

Figure 8. *Post-mortem* observation of internal tissues in *Oreochromis niloticus* fed 1.5% powdered *Aloe barbadensis* and challenged by intraperitoneal injection with *Pseudomonas aeruginosa*. a) Pale liver and pancreas with gallbladder darkening, b) hemorrhagic organs, and c) uninfected fish (n = 3 fish per treatment).

Similarly, comparable studies such as those of El-Houseiny et al. (2022), who incorporated parsley seed, and Montaser et al. (2021), who supplemented the diet of Nile tilapia with *Boswellia serrata*, reported higher survival rates in plant-treated groups than in the control group, with RPS values exceeding 33.33%. These authors attributed such responses to the action of bioactive compounds, including flavonoids, terpenoids,

tannins, saponins, and anthraquinones, which enhance antimicrobial, antioxidant, and immunostimulatory activity in fish.

Even in the absence of direct immunological measurements, the results obtained here suggest a potential antimicrobial effect of *A. barbadensis* and a possible stimulation of the innate immune system of *O. niloticus*. However, this would require further investigation to clarify the underlying mechanisms (Khan et al. 2022).

In this regard, Shehata et al. (2013) reported that thyme extract (*Thymus vulgaris*) at 0.25% exhibited antimicrobial activity against *P. aeruginosa*, an effect attributed to compounds such as carvacrol, thymol, γ-terpinene, and *p*-cymene, which exert inhibitory activity through interactions with the bacterial cell membrane. Likewise, *Spirulina platensis* and *Salvia officinalis* improved immune response and resistance to infection owing to their high content of proteins, antioxidants, essential amino acids, fatty acids, carotenoids, vitamins, and minerals (Abdellatief et al. 2018). Other studies have further shown that supplementation with *Silybum marianum* combined with coenzyme Q10 (Khalil et al. 2022) and guava leaf extract (Hossain et al. 2024) increased survival following challenge with *P. aeruginosa*; such effects are mainly associated with their antimicrobial and antioxidant properties.

Accordingly, these findings support the effectiveness of medicinal plants in managing diseases caused by *P. aeruginosa*, highlighting their potential as sources of novel antimicrobial agents. They also underscore that their efficacy depends on concentration, exposure time, route of administration, and the physiological condition of the host; in other words, the minimum effective concentration should be considered the optimal dose leading to maximal antimicrobial activity (Awad & Awaad 2017, Stratev et al. 2018, Angelini 2024). In this regard, the cited literature demonstrates that prophylactic treatments administered days before infection can significantly reduce mortality compared with conventional treatments, thereby lowering production costs and environmental impact by decreasing antibiotic use (Imtiaz et al. 2023).

CONCLUSIONS

The results indicate that powdered *A. barbadensis* has potential as a prophylactic dietary additive in Nile tilapia, as it showed no adverse effects on survival and suggested a possible immunoprotected effect against *P. aeruginosa*. Its evaluation via both immersion and intraperitoneal challenge demonstrated a favorable effect on survival under both routes of infection. Overall, these findings support its potential as a preventive alternative against bacterial diseases in cultured fish and its possible contribution to reducing antibiotic dependence in aquaculture. However, further studies on immunological, toxicological, and productive parameters, as well as commercial-scale validation, are still required.

Credit author contribution

L.T. Portillo-Delgado: original draft, writing, methodology, formal analysis, review and editing; J.V. Trejo-Flores: original draft, writing, methodology, formal analysis, review and editing; V. Peraza-Gómez: original draft, writing, conceptualization, validation, supervision, project administration, formal analysis, review and editing; L. Arias-Rodríguez: review and editing; A.I. Campa-Córdova: review and editing; A. Luna-González: review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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