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Aloe vera (L.) Burm.f. enhances caudal fin regeneration in zebrafish (*Danio rerio*)

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ABSTRACT

Zebrafish are widely recognized for their ability to completely regrow their caudal fins following an amputation injury. This makes them an ideal model organism for investigating the wound-healing potential of various medicinal plants. In this study, the regenerative effect of *Aloe vera* (L.) Burm.f. extracts on the amputated caudal fins of zebrafish was evaluated using three extract concentrations (i.e., 0.3%, 0.6%, and 1.2%) applied per 1.3 L of water in the tank. Results showed that *A. vera* extracts contributed to the survivability of wounded zebrafish and the regeneration of their caudal fins compared with untreated wounded zebrafish. Among the extract concentrations applied, 0.6% yielded a regeneration rate of 0.19 mm/day and an endpoint caudal fin regeneration percentage of 99.17% within 14 days of application, with no recorded mortality. The results suggest that *A. vera* is a promising candidate for wound healing and the regeneration of damaged tissues in zebrafish. Furthermore, it indicates its potential use by fish pet owners and businesses as an alternative to commercial fish wound-healing products.

Keywords: medicinal plants, tissue regeneration, survivability, wound-healing

INTRODUCTION

There is increasing interest in exploring the regenerative potential of plant extracts in fishes with damaged fins due to the growing demand for sustainable solutions in the aquaculture industry. Fins, specifically the caudal fins, produce propulsive force, which is essential for locomotion, balance, and survival in fish (Liu et al. 2020). The loss of this vital part can result in decreased growth rates, reduced reproductive success, and increased mortality, thereby negatively impacting the fish industry. Existing commercial treatments for fin damage can be expensive and may have adverse environmental effects. Meanwhile, plant extracts possess potential therapeutic properties, which make them promising alternatives for improving overall fish health, increasing wound-healing capabilities, and reducing possible environmental impacts (Ayisi et al. 2025).

Traditional herbal plants are widely recognized as cost-effective wound healing treatments. *Aloe vera* (L.) Burm.f. is known to possess antibacterial, antiseptic, and antiviral properties that serve as a basis for investigating its potential as an all-natural fish wound treatment. In addition, it has demonstrated regenerative properties. For instance, Hekmatpou et al. (2019) reported that its regenerative properties are attributed to the compound glucomannan, which is rich in polysaccharides such as mannose.

Aloe vera is a succulent plant that belongs to the family Asphodelaceae and is commonly found in arid regions of Africa, Asia, Europe, and America (Surjushe et al. 2008). The leaf of this plant is fleshy, triangular in shape, and has serrated edges. It consists of three layers. The first layer comprises an inner gelatinous liquid that contains 99% water, while the remaining 1% consists of glucomannans, amino acids, lipids, sterols, and vitamins. This layer is commonly extracted for skin and hair care applications, as it effectively cleanses the hair shaft by removing excess oil and residue from previously applied hair products. When used regularly, it may also promote hair growth and improve hair texture. The middle layer, on the other hand, consists of latex which is the bitter yellow sap rich in anthraquinones and glycosides. Lastly, the outer layer, called the rind, serves as protection for the inner layers and helps in the synthesis of carbohydrates and proteins (Jadhav et al. 2020).

In addition to the moisture and hydration effects of *A. vera* on the skin, it also provides medicinal benefits attributed to its antibacterial, antiviral, and antiseptic properties, which help treating wounds and other skin conditions. According to Surjushe et al. (2008), it contains 75 active compounds, including vitamins A, B, C, E, B12, folic acid, and choline, which function as antioxidants that neutralize free radicals. Additionally, enzymes such as alkaline phosphatase, amylase, bradykinesia, carboxypeptidase, catalase, cellulase, lipase, and peroxidase facilitates sugar and fat breakdown. Minerals such as calcium, chromium, copper, selenium, magnesium, manganese, potassium, and zinc also act as antioxidants that serve a variety of metabolic roles, specifically in various metabolic pathway.

Given the existing reports on the phytochemicals and wound-healing capacity of *A. vera*, it is hypothesized that it may also exhibit a regenerative activity essential for the recovery of fishes from caudal fin injuries. Thus, this study aimed to investigate the regenerative effect of *A. vera* in zebrafish. Specifically, it aimed to determine the survivability rate of zebrafish following exposure to varying concentrations of *A. vera* extract; to evaluate the regenerative effects of varying concentrations of *A. vera* extract in terms of morphological caudal fin changes; to determine the rate and percentage of caudal fin tissue regeneration in zebrafish exposed to varying concentrations of *A. vera* extract; and to identify the most effective concentration of *A. vera* extract for caudal fin tissue regeneration in zebrafish.

METHODS

Research Design

The testing of the sample extract was conducted using an experimental approach to compare and contrast the regenerative activity of varying *A. vera* concentrations based on caudal fin growth in zebrafish.

Seven aquarium tanks were assigned to different treatments: T_1, wounded zebrafish with 0.3% *A. vera* extract; T_2, wounded zebrafish with 0.6% *A. vera* extract; T_3, wounded zebrafish with 1.2% *A. vera* extract; T_4, wounded zebrafish with a commercial fish wound medicine (Obat Ikan); T_5, wounded zebrafish with no treatment; T_6, non-wounded zebrafish with 0.6% of *A. vera* extract; and T_7, non-wounded zebrafish with no treatment. The positive control was T_4, while the negative controls were T_5, T_6, and T_7. This study consisted of three trials, each comprising three replicates (Figure 1).

Sample Collection

Mature leaves of *A. vera* plant were obtained from a commercial garden located in Silang, Cavite. Meanwhile, adult male zebrafish measuring 3 to 4 cm and aged 12 to 18 months were purchased from a local pet supplier in the same municipality. Zebrafish are typically considered to be in optimal condition between 3 to 18 months of age (Avdesh et al. 2012). In this study, 12- to 18-month-old zebrafish were selected because they possess larger caudal fins as compared to younger ones, making them suitable for amputation procedures. Moreover, the ability of injured caudal fins to fully regenerate decreases with age (Shao et al. 2011), which was considered to ensure reliable measurement of mortality and regeneration rates. In addition, male zebrafish were used to eliminate the risk of mating and reproduction. Male zebrafish could be distinguished from females by their more slender bodies, darker coloration, and yellow anal fin (Avdesh et al. 2012).

Habitat Set Up and Maintenance

Five zebrafish were placed in each aquarium tank measuring $4.5 \times 3.0 \times 6.0$ in^3. Each tank was filled with 1.3 L of water based on standard tank volume calculations. White stones (250 g per tank) were added to each aquarium tank to help boost algal growth, which served as additional food source and helped mimic the natural habitat of zebrafish. Fish tank aerators were used to provide sufficient oxygen and were set to create a mild water current preferred by zebrafish prefer (Lawrence and Mason 2012). In addition, a centralized light system was operated for

12 h daily to maintain a good habitat and environmental conditions. The tank water was partially replaced on the second, fourth, sixth, eighth, 10th and 12th days prior to treatment application and caudal fin measurement using an aquarium siphon. During replacement, a partial water change method was employed, as it is less stressful for fish and preserves beneficial biological communities (Pelletier et al. 2020). Water with a height of one inch (i.e. 25%) was retained in the tank to ensure zebrafish survival. Stock tap water was then added to the tank as a part of the replacement procedure. Temperature and pH level were maintained at 24°C and 8.17 respectively (Brand 2014), and were monitored after each water replacement and treatment application to prevent physiological stress, reduced growth, disease, or mortality (Jorge et al. 2023).

Preparation of the *A. vera* Plant Extract

Fresh *A. vera* leaves were thoroughly washed, placed upright for 10 min to allow the yellow-tinted resin to drain, and then peeled to remove the thick outer skin. The inner gel was scooped and blended to achieve a liquid consistency (Shoemaker 2019). The mixture was strained to separate the gel and liquid extract, 55% crude yield on average. This crude gel extract was used for all treatments. In this study, concentration is referred to the proportion of crude *A. vera* extract added to the water tanks rather than the concentration of a specific isolated active compound. Although *A. vera* contains several bioactive constituents such as glucomannan, anthraquinones, and flavonoids, the present study did not quantify these individually.

***Aloe vera* Extract Application**

Twelve aquarium tanks (four per replicate) received different amounts of *A. vera* extract on the first, second, fourth, sixth, eighth, 10th and 13th days. Three concentration treatments were applied: 0.3%, 0.6%, and 1.2% (v/v), following Khanal et al. (2012). These concentrations were prepared by adding 4 mL, 8 mL, and 16 mL of crude *A. vera* extract, respectively, to 1.3 L (1,300 mL) of water per tank, corresponding to 0.3%, 0.6%, and 1.2% of the total solution volume. Additionally, 1 mL of commercial fish wound medicine, known to treat fish diseases by acting as an anti-bacterial, anti-rot, and antifungal agent, was also applied to tanks devoted to the positive control during the same application schedule.

Zebrafish Acclimation and Feeding Strategy

The floating bag acclimation method was used to acclimatize the zebrafish. The plastic bag containing the zebrafish was placed on the water for 1 h (Dhanasiri et al. 2013). After the acclimation period, zebrafish were given 24 h to adapt to their respective tanks prior to the application of the treatments.

The zebrafish in each aquarium tank were fed with Maxflo dry fish feed once a day in the morning to promote intestinal emptying and avoid the risk of overfeeding. Most fish need at least 16 h or one whole day to fully digest the food they consume (Sharpe 2024). Overfeeding the fish may result in indigestion and the uneaten food may become excess waste that can be a risk to the fish. In addition, the excess food waste may lead to higher ammonia levels in the water and may eventually lead to serious health consequences for the zebrafish.

Zebrafish Caudal Fin Amputation

Each zebrafish was placed on a Petri dish, and the caudal fin was amputated distal to the end of the bony musculature using a sterile razor blade. The cut was positioned at the terminal portion of the caudal fin, as incisions made close to the musculature may increase the risk of mortality (Haney et al. 2021).

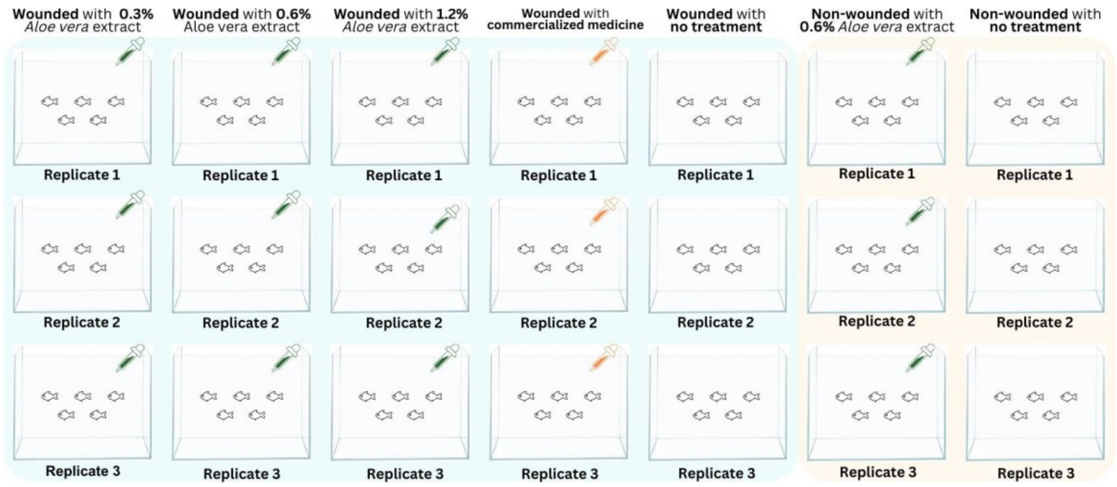

Figure 1. An illustration showing the experimental setup for each trial.

The amputation procedure for each zebrafish was completed within approximately two minutes to minimize handling time and reduce physiological stress associated with prolonged removal from water (Wallace et al. 2018). Caudal fin clipping was a commonly employed method in zebrafish regeneration studies due to the species' high regenerative capacity and the minimally invasive nature of distal tissue removal. All procedures were conducted in a Bureau of Animal Industry-accredited veterinary diagnostic laboratory under the supervision of a licensed veterinarian, ensuring compliance with animal welfare standards.

Wound Exposure to Treatments

After the amputation, the wounded zebrafish was placed back in its aquarium. Then, after 30 min, the *A. vera* extracts and the commercial fish wound medicine were administered in their specific aquarium tanks to allow the exposure of the wound to the treatments. The whole observation period for the caudal fin regeneration was conducted over 14 days (Sehring and Weidinger 2020). The zebrafish was exposed to the different *A. vera* extract concentrations and other treatments seven times throughout the observation period. During each partial water replacement, approximately 25% of the original tank water was retained, and the designated concentration of *A. vera* extract and the fish wound medicine were re-applied to the restored tank volume. This procedure approximated the target treatment concentration, although some residual extract from the previous dosing was unavoidably retained. In cases of mortality, the dead fish was isolated immediately to avoid spread of contamination.

Data Gathering Procedure

Both qualitative and quantitative data were gathered daily for 14 days. Caudal fin measurement was done during nighttime as zebrafish exhibit reduced tail mobility in the evening (Kalueff et al. 2013). The initial length of the zebrafish prior to the amputation was recorded in a data sheet. After the amputation, the initial length on the remaining tail was measured, as well as the development of their length on the third, fifth, seventh, ninth, 11^{th}, and 14^{th} days. To identify the length of the regenerated caudal fin, each zebrafish was placed on a convex Petri dish, which had an attached measuring device to measure the progress in millimeters. This procedure was also done on the first, third, fifth, seventh, ninth, 11^{th}, and 14^{th} days of the observation period.

Data Analysis and Statistical Treatment

Statistical data analysis was used to determine the survivability rate of the zebrafish after exposure to the varying concentrations of *A. vera* extract, and the percentage of caudal fin tissue regeneration in zebrafish exposed to varying concentrations of *A. vera* extract.

To determine the survivability rate, this study used frequency counts and percentages, wherein the number of live zebrafish at the end of the observation period was counted and divided by the total number of zebrafish at the beginning, then multiplied by 100. The average daily regeneration rate of the caudal fin, which was determined to assess the rate of tissue regrowth over time, was calculated by dividing the difference between the final fin length and the initial fin length by the total number of observation days minus one. This provided an estimate of the mean daily increase in fin length across the observation period. On the other hand, to determine the regeneration percentage of the caudal fin, the average final length was divided by the initial length of the caudal fin, then multiplied by 100.

The data were also analyzed using appropriate statistical tools to identify the regenerative effect of *A. vera* and provide possible recommendations. Tests for normality and homogeneity of variances were first conducted to ensure the validity of the statistical comparisons. Based on these assumptions, suitable parametric or non-parametric tests were applied. Bivariate statistics and a chi-square test for independence were employed using the Statistical Package for Social Science (SPSS) program, version 25.

RESULTS

Survivability Rate

The survivability rate of zebrafish in each treatment was determined by counting the number of surviving fish in each tank after 14 days of treatment application. As shown in Table 1, a 100% survivability rate was observed in all treatments with *A. vera* extract (T_1, T_2, T_3, and T_4), indicating the extract's positive effect on survival following caudal fin amputation. In contrast, the untreated control group (T_5) exhibited a lower survivability rate of 77.77%, suggesting that the absence of *A. vera* treatment may have increased the mortality risk in wounded zebrafish.

Table 1. Survivability of the zebrafish 14 days after exposure to different treatments.

Treatment	Survivability Rate (%)
T_1	100 ± 0.00
T_2	100 ± 0.00
T_3	100 ± 0.00
T_4	100 ± 0.00
T_5	77.77 ± 19.44
T_6	100 ± 0.00
T_7	100 ± 0.00

Regenerative Effects of *A. vera* in Zebrafish

The regenerative effects of *A. vera* extract on zebrafish caudal fins after amputation showed progressive changes over the observation period (Figures 2 and 3). On day one, images displayed caudal fins after amputation. By day three, no significant growth progression, changes in shape or size, or blastema formation were observed. However, by day five, notable changes were evident in treatments T_1, T_2, T_3, and T_4, with increased fin thickness and hardening, while T_5 showed no such changes. The caudal fins of T_1-T_4 also exhibited a color change, with the bright original color fading due to a thin white slime developing on the surface, likely contributing to pathogen protection. Additionally, a distinct blastema line appeared, demarcating old fin tissue from the regenerating areas.

On day seven, the caudal fins began to regain their original thickness, though their color appeared more faded than on day five. By day nine, the regenerating fin tips had begun to approximate the shape and size of the original fin, with the blastema line gradually fading and blending with the new tissue. By day 11, fins in T_1, T_2, and T_3 had an almost translucent appearance, indicating the continued formation of new tissue. By day 14, caudal fin regeneration in all treatments closely resembled the original fin shape, size, color, and ray formation, marking substantial recovery.

Regeneration Rate and Endpoint Regeneration Percentage in Zebrafish

The average daily regeneration rate of amputated caudal fins ranged from 0.14 to 0.19 mm/day across treatments (Table 2). Zebrafish exposed to T^2 exhibited the highest regeneration rate (0.19 ± 0.59 mm/day), while those in T^3 showed the lowest (0.14 ± 0.40 mm/day). Treatments 1, 4, and 5 recorded comparable values, ranging from 0.15 to 0.16 mm/day. These findings indicate that, although differences were relatively small, T_2 showed a slight advantage in promoting caudal fin regrowth compared with the other treatments.

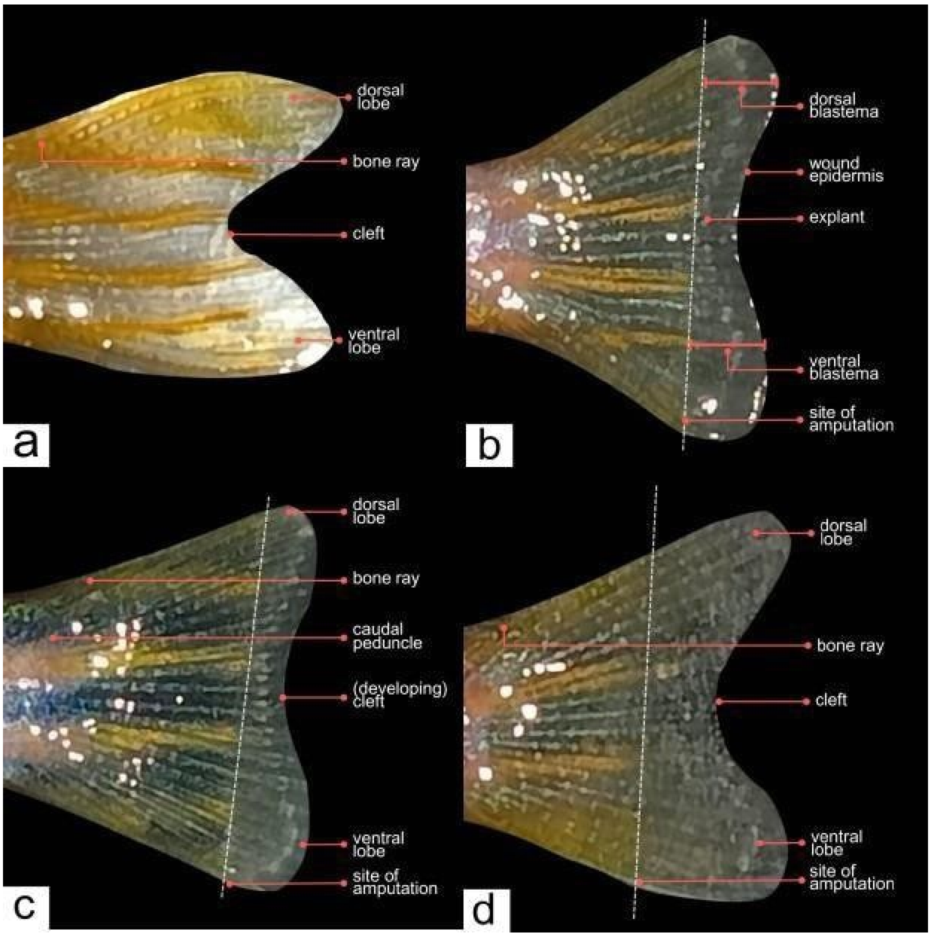

Figure 2. Morphology of (a) non-amputated caudal fin, (b) caudal fin with blastema formation, (c) regenerated caudal fin structure and (d) final regenerated caudal fin structure. Images shown are representative samples selected to highlight the morphological changes observed.

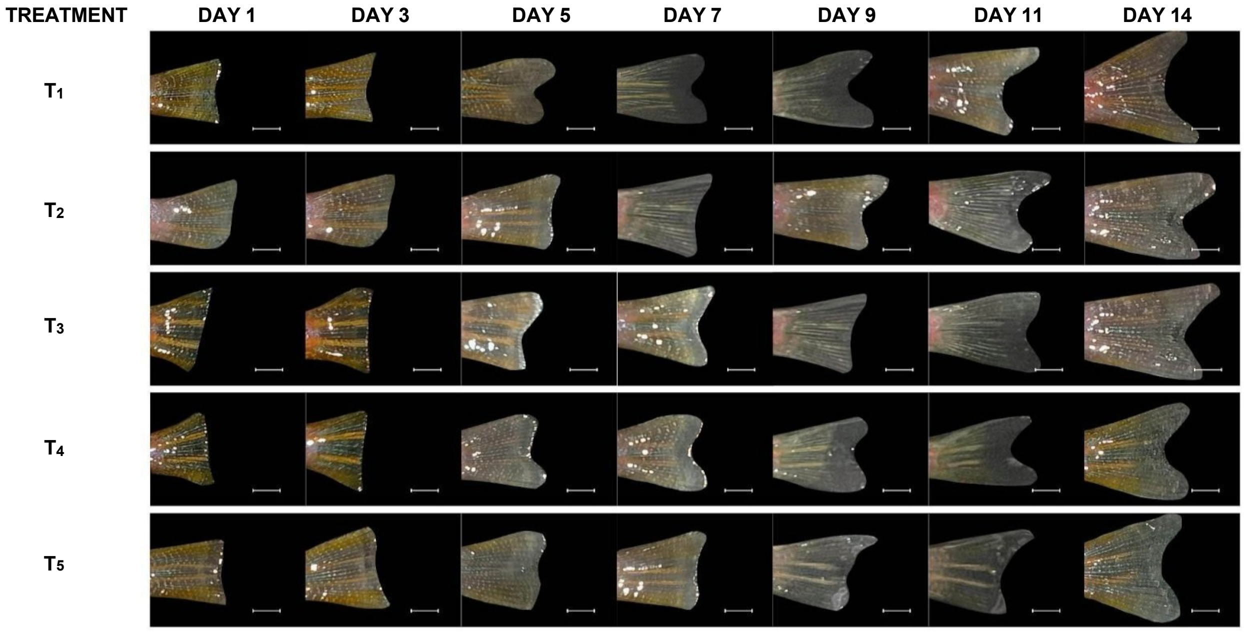

Figure 3. Regeneration progresses of amputated caudal fins within 14 days of exposure to each treatment. Reference size: 1 mm. Note: T_6 and T_7 are not shown as no amputation was done on the zebrafish. Images shown are representative samples selected to highlight the morphological changes observed.

Table 2. Average regeneration rate (mm/day) of amputated caudal fins exposed to different treatments.

Treatment	Regeneration Rate (mm/day)
T_1	0.16 ± 0.39
T_2	0.19 ± 0.59
T_3	0.14 ± 0.40
T_4	0.16 ± 0.36
T_5	0.15 ± 0.44

The caudal fin regeneration percentage was measured at the end of the observation period and compared with the fin length prior to amputation. As shown in Table 3, the highest endpoint regeneration percentage (99.17%) was observed in zebrafish treated with 0.6% *A. vera* extract (T_2), which was significantly higher than the positive control (95.39%). Zebrafish treated with 1.2% *A. vera* (T_3) also showed a notable regeneration percentage (93.24%), though this was not significantly different from the positive control, indicating its potential as an effective regenerative treatment. The commercial wound treatment (T_4) resulted in a similar regeneration percentage to the positive control, at 95.39%. In contrast, the untreated group (T_5) showed the lowest regeneration percentage (87.24%), which was not significantly different from that of zebrafish treated with 0.3% *A. vera* (T_1), which had an 85.80% regeneration percentage. These results highlighted 0.6% *A. vera* as the most effective concentration for promoting caudal fin tissue regeneration in zebrafish.

Table 3. Endpoint regeneration percentage of amputated caudal fins after 14 days of exposure to different treatments. Means followed by the same letter indicate no significant difference at 0.05 level of significance.

Treatment	Regeneration Percentage
T_1	85.80[c]
T_2	99.17[a]
T_3	93.24[b]
T_4	95.39[b]
T_5	87.24[c]

DISCUSSION

Survivability Rate

The 100% survivability observed in the tanks treated with *A. vera* extract may be attributed to the diverse phytochemicals present in the plant, including saponins, anthraquinones, flavonoids, alkaloids, and phenols. These compounds collectively exhibit properties beneficial for wound healing and infection prevention. Saponins, anthraquinones, and phenols have well-documented antibacterial activities, which could have contributed to the inhibition of pathogenic bacteria in the zebrafish tanks, thus reducing infection risks associated with open wounds (Sayhan et al. 2017). Additionally, alkaloids possess analgesic and local anesthetic properties, potentially reducing pain and stress in the injured zebrafish, which can support recovery by lowering physiological stress responses (Sayhan et al. 2017). Flavonoids, known for their role in regulating cell growth and anti-inflammatory effects, could further aid in tissue repair by modulating cellular responses around the wound site. Together, these bioactive compounds likely contributed to the 100% survivability observed, supporting the potential efficacy of *A. vera* in reducing mortality related to caudal fin injuries.

In contrast, the tanks with wounded zebrafish that received no treatment experienced mortality, indicating that *A. vera* extract may have a protective effect against mortality in injured zebrafish. These findings align with previous studies demonstrating *A. vera*'s therapeutic properties, which include wound healing and infection control in aquatic animals (Subramanian et al. 2006; Hekmatpou et al. 2019).

Regenerative Effects of *A. vera* in Zebrafish

The presence of a white slime on the regenerating caudal fin in zebrafish treated with *A. vera* extract suggests an increase in the natural slime coat. This slime coat acts as an additional protective barrier against infection, which may enhance tissue regeneration by reducing the likelihood of pathogenic invasion in the affected area (Subramanian et al. 2006). *Aloe vera* extract has also been reported to stimulate collagen synthesis and collagen cross-linking in injured tissues, accelerating wound healing and supporting the structural integrity of the new tissue (Hekmatpou et al. 2019).

Tannins, another key component of *A. vera*, are known to enhance blood clotting, which supports the formation of new tissue in the wounded area by promoting coagulation and tissue stabilization (Chokotho 2005). This could have played an essential role in accelerating caudal fin regeneration. In addition to tannins, saponins, phenols, and anthraquinones present in *A. vera* offer a range of protective properties, including anti-inflammatory, antibacterial, antifungal, and antiviral effects, which can mitigate complications and reduce the risk of infection in the regenerating fin. These combined effects likely aided cell and tissue repair processes, promoting the swift regeneration observed in the caudal fins.

Interestingly, flavonoids in *A. vera* have been noted for their stress-reducing effects due to their polyhydroxylated aromatic structures, which may have helped the zebrafish manage physiological stress during the amputation and subsequent measurement phases. The antioxidative effects of flavonoids could mitigate oxidative stress, further supporting a conducive environment for tissue regeneration (Hekmatpou et al. 2019).

Regeneration Rate and Endpoint Regeneration Percentage in Zebrafish

The regeneration rate analysis supports the potential of *A. vera* in enhancing fin regrowth, as zebrafish treated with 0.6% extract (T_2) exhibited the fastest daily regeneration compared with the other treatments. Although the differences across treatments were relatively small, this trend highlights that *A. vera* can accelerate the pace of tissue repair, not only increasing the extent of regeneration but also reducing the time required for recovery. This suggests that the previously mentioned phytochemical constituents of *A. vera* may act to stimulate cellular processes associated with rapid wound closure and tissue regrowth. The slower regeneration observed in the 1.2% group (T_3) reinforces the idea that an optimal concentration threshold exists, beyond which the stimulatory effects may be diminished.

In terms of the endpoint regeneration percentage, the observation that T_3 (a higher concentration of *A. vera*) exhibited a lower regeneration percentage than T_2 may be due to variations in tank pH levels. Treatment 3 tanks had a slightly acidic mean pH of 7.84, while T_2 tanks maintained a more basic pH of 8.32. Since pH can influence enzyme activity and nutrient absorption in zebrafish, a more acidic environment may have hindered optimal conditions for tissue regeneration. Furthermore, the performance of T_1, which resulted in a regeneration rate comparable to untreated wounded zebrafish, may indicate that this concentration of *A. vera* was insufficient to exert its phytochemical effects fully. Research has demonstrated that higher concentrations of bioactive compounds improve absorption and efficacy at wound sites, supporting the notion that a threshold concentration may be necessary for effective treatment (Soni et al. 2019).

By identifying the treatment with the highest caudal fin regeneration percentage, fastest regeneration rate, and survivability over the 14-day observation period, the most effective concentration of *A. vera* was determined. T_2, with a 0.6% *A. vera* concentration, resulted in the highest regenerated caudal fin tissue, significantly surpassing the positive control. Additionally, T_2 achieved a 100% survivability rate, making it the optimal concentration for both survival and tissue regeneration in wounded zebrafish. These results highlight the potential of using a 0.6% *A. vera* concentration as an effective, natural therapeutic agent for promoting caudal fin regeneration and overall survivability in zebrafish.

In conclusion, *A. vera* extract demonstrated potential in enhancing survivability and promoting tissue regeneration in zebrafish with amputated caudal fins. Tissue regeneration began with blastema formation, and the presence of a white slime coat by day five suggested an enhanced barrier against pathogens due to *A. vera*'s natural protective properties. Among the tested concentrations, 0.6% was the most effective, yielding the highest caudal fin regeneration rate and surpassing the outcomes of commercial fish wound treatments, as confirmed by post-hoc analysis. These findings support the potential use of *A. vera* extract, particularly at 0.6%, as an effective natural remedy to enhance wound healing and tissue regeneration in zebrafish. Although the phytochemicals of *A. vera* are well studied, future research should focus on quantifying these constituents in crude extracts to improve reproducibility and enable more precise comparisons across studies.

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GENERATIVE AI STATEMENT

During the preparation of this work, the authors used ChatGPT-4 to improve language and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of this publication.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with ethical guidelines for the care and use of animals in research. It has undergone review and approval by the Ethics Review Board of Cavite State University. To meet the recommendations set by the Institutional Animal Care and Use Committee (IACUC), the study was conducted in a research facility accredited by the Bureau of Animal Industry. Efforts were made to minimize animal suffering, especially during fin amputation.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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