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Lyophilized *Terminalia catappa* Leaf Extract: A Natural Algicide, Improves Survival of *Poecilia reticulata* in Aquaria

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Abstract. The search for plant-derived solutions as an alternative to synthetic aquarium water conditioners has driven interest in aquaculture due to the linkage of poor water management to environmental degradation and ecosystem imbalance. This study assessed the effectiveness of lyophilized *Terminalia catappa* leaf extract as a natural algicide and water conditioning agent in aquaria containing *Poecilia reticulata.* Three concentrations (500, 1000, 1500 ppm) were tested, alongside negative and positive controls during the 30-day experiment. Throughout the study, water quality parameters, algal proliferation, and fish survival were monitored. The treatment groups exhibited improved survival, compared to the untreated control. 1000 and 1500 ppm significantly delayed and reduced algal growth. The extract stabilized water conditions and maintained water quality parameters within biologically acceptable ranges. These findings demonstrate the efficacy of lyophilized *T. catappa* leaf extract as an eco-friendly and sustainable alternative to conventional water conditioners. This study can benefit both environmental health and resource-efficient fish production by promoting plant-based strategies that reduce reliance on synthetic chemicals. Additionally, this study supports the United Nations' Sustainable Development Goals: SDG 6 (Clean Water and Sanitation) by improving water and quality management, SDG 12 (Responsible Consumption and Production) through the use of natural and biodegradable materials, and SDG 14 (Life Below Water) by minimizing ecological risks associated with chemical pollutants in aquatic systems.

Keywords: algicide; aquaculture; *Poeccilia reticulata*; *Terminalia catappa*; water quality

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INTRODUCTION

The ornamental fish industry represents the highest value industry globally, yet sustainable water management remained insufficiently explored (Pountney, 2023), and as a significant sector of aquaculture, proper aquarium maintenance is required in order to achieve optimal water quality that is essential for the survival, health, and reproduction of the fish, since aquatic species are highly sensitive to fluctuations in physicochemical parameters (Rashid *et al*., 2022). Water quality plays a vital role in maintaining and supporting fish well-being and other aquatic organisms, particularly in tank conditions. The common problem encountered in aquarium keeping is the accumulation of metabolic wastes, excess feed, algal growth, and microbial activity, all of which can deteriorate water quality, so proper monitoring and management are highly needed (Yusoff *et al*., 2024). Water parameters can jointly influence aquatic organisms, fish health, and stability of the system (Hridoy *et al*.,2025).

Previous studies showed that the utilization of aquatic plants improves water quality, can regulate microbial activities, and enhance fish behavior showing that biological approaches is effective in water conditioning and thus supporting the use of natural solutions in aquatic systems (Xu *et al*., 2026). The utilization of plant-based extracts is said to influence water chemistry and fish physiology, including stress reduction and antimicrobial activity due to the presence of bioactive compounds, tannins, flavonoids and alkaloids (Aiswarriya *et al*., 2024). The use of conventional techniques is commonly followed in these modern days, which are often equipment-dependent (Banerjee, 2025; Zuhaer *et al*., 2025), but due to the linkage of poor water management to ecosystem imbalance and environmental degradation (Tahiludin *et al*., 2025), the search for natural alternatives has gained attention in the aquaculture industry.

Among the ornamental fish, guppy or *P. reticulata* is commonly used as a model organism in experimental studies because they are easy to maintain, has a short life cycle but has high reproductive capacity, and is sensitive to environmental changes, making it particularly suitable in genetic studies, aquatic ecology, and toxicology (Nakajima & Taniguchi, 2001; Imai *et al*., 2022). To maximize productivity and ensure long-term sustainability of aquaculture systems, optimum water quality maintenance is essential, as water quality is increasingly influenced by anthropogenic pressures, including pollution and intensive water use (Tumwesigye *et al*., 2022; Bamaniya *et al*., 2024), and it varies temporally and spatially (Boyd, 2020). Amidst these challenges, occurring in both natural and artificial aquatic systems, is the excessive algal growth, which is one of the most persistent water quality problems.

It often leads to harmful algal blooms (HABs) that reduce dissolved oxygen levels and release harmful toxins that can be detrimental to aquatic organisms (Rolton *et al*., 2022). Failure to maintain key conditions such as temperature and lighting can adversely affect the system (Gómez *et al*., 2024; Anaswara *et al*., 2024) and in addition to environmental stressors that can alter fish behavior and physiological processes, there are pharmaceutical contaminants and salinity changes that may reduce organismal performance, leading to potential ecological instability (Brookhart *et al*., 2025). In order to achieve proper fishkeeping, the essential requirements should be met, such as good water quality, stable environmental conditions, and controlled stocking density (Bierbach *et al*., 2025).

In controlling algal proliferation, chemical algicides are readily available and commonly utilized; however, their misuse can negatively affect aquatic ecosystems and fish well-being (Giang *et al*., 2023). Accordingly, the search for eco-friendly and plant-based alternatives has drawn increasing interest. *T. catappa*, commonly known as Talisay in the Philippines, has gained growing attention due to its beneficial application in aquaculture. They are known to simulate natural black water environments, which are favorable to Guppies, and contains tannins and humic substances capable of lowering pH and preventing pathogenic microorganisms, supporting its capacity to stand as a natural water conditioner. Studies reported its effectiveness in water quality improvement in tilapia farming ponds (Giang *et al*., 2023), enhancement of fish larvae survival and growth (Ramanan S *et al*., 2024), and maintenance of Betta or Siamese fighting fish tanks (Malawa *et al*., 2022). Talisay has been widely utilized and studied for its bioactive compounds, including flavonoids, phenolics and tannins, which exhibit antioxidant, antimicrobial, and anti-algal properties (Hung *et al*., 2022; Wang *et al*., 2024).

However, its application as both a water conditioner and algicide in ornamental aquaculture remained limited and unexplored, despite the advancements available in the fish industry. Talisay leaf extract has been investigated primarily using aqueous or crude extract rather than lyophilized preparations (Chansue & Assawawongkasem, 2008), and studies relating to this mainly focused on Betta fish and other ornamental fish species, with limited investigations involving Guppies or *P. reticulata* (Roanisca *et al*., 2016), and lastly, comparative studies between natural extracts and commercial water conditioners are still lacking (Evangelista-Barreto *et al*., 2025). This study would like to address these gaps by evaluating the efficacy of lyophilized *T. catappa* leaf extract as a natural water conditioner, controlling algal growth, and improving the survival of Guppy or *P. reticulata*. It specifically 1) determines the effects of lyophilized *T. catappa* leaf extract on water quality parameters, including pH level, salinity, and dissolve oxygen; 2) assesses the survival rate of *P. reticulata* under different extract concentrations; 3) evaluate the anti-algal activity of the extract through qualitative and quantitative analysis of algal growth; 4) evaluates comparison of the efficacy of *T. catappa* extract with commercial water conditioner "Aqua Care solution" and lastly, 5) identifies the optimal concentration of *T. catappa* leaf extract that balances algal inhibition, water quality and fish survival.

METHODS

Experimental Design

This study used a completely randomized design, consisting of five experimental groups: the negative control, positive control (treated with Aqua Care solution), and the treatment groups (treated with the lyophilized *T. catappa* leaf extract) at concentrations of 500 ppm, 1000 ppm, and 1500 ppm, each of which was set in triplicate.

Preparation of the Extract

Mature *T. catappa* leaves were collected, cleaned, air-dried, and subjected to lyophilization. The dried material was pulverized and soaked in distilled water (50 g per 2 L) for 72 hours. The mixture was filtered and aerated for 72 hours to obtain the final extract at room temperature. The final extract was filtered again using a 100 µm sieve to produce the lyophilized *T. catappa* leaf aqueous extract used in the experiment. The three concentrations of the extract in this study were modified based on the study of Giang *et al*. (2023). The concentrations for the different treatments were 500, 1000, and 1500 ppm with 2 mL, 4 mL, and 6 mL of the extracts per 4L water, respectively.

Fish Husbandry

The stocking density of the Guppies was computed based on the study of Görelşahin *et al.* (2018). Male *P. reticulata* were maintained at a stocking density of 5 fish per 1.67 L (a proportional calculation where one Guppy fish requires 0.33 L multiplied by 5 fish) per tank under controlled laboratory conditions for 30 days. Fifteen uniform rectangular tanks were used for all the treatments with proper aeration.

Measurement of Physicochemical Parameters

Water quality parameters were measured once every 5 days at 8:00 AM throughout the experiment. All instruments were calibrated before each use following manufacturer specifications. Dissolved oxygen (DO) was measured using a digital DO meter, while pH, salinity, and temperature were measured using a multiparameter water quality meter.

Assessment of Fish Survival

Fish survival was monitored and recorded daily. The survival rate of *P. reticulata* was calculated using the formula by Sultana *et al.* (2018):

$$\text{Survival rate (\%)} = \frac{No.of\ fish\ alive}{No.of\ fish\ used\ or\ stocked} x100$$

Assessment of Algal Growth

In order to qualitatively and quantitatively evaluate the algal proliferation across the experimental groups, visual inspection and algal density calculation were done. The density calculation was based on Fuentes (2017).

Data Collection

Water quality parameters (pH, temperature, and dissolved oxygen) were monitored at regular intervals. Algal density was determined using hemocytometer counts, while survival was recorded daily.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test, and a chi-square test with significance set at $p < 0.05$. Descriptive statistics (mean ± standard deviation) were used to summarize water quality parameters and survival data.

RESULTS AND DISCUSSION

The effects of lyophilized *T. catappa* leaf extract on water quality parameters

Across treatment groups, the water quality parameters remained within acceptable ranges (Table 1). The pH levels showed a decreasing trend in the treatment groups (with Talisay leaf extract), but only a slight reduction was observed (Figure 1). The dissolved oxygen (DO) Levels remained relatively stable compared to the negative control. The stabilization of dissolved oxygen in treated groups suggests reduced biological oxygen demand, likely due to controlled algal growth. This can be supported by the findings reported by Giang *et al*., (2023), where *T. catappa* extract improved water quality by limiting microbial and algal activity.

Table 1. Water Quality Parameters Across Experimental Groups (Mean ± SD)

Values are expressed as mean ± standard deviation (n = 3 replicates per treatment).

Group	pH	Salinity (ppt)	Dissolved Oxygen (mg/L)	Temperature (°C)
Negative Control	7.92 ± 0.10	0.16 ± 0.05	4.10 ± 0.30	27.06 ± 0.20
Positive Control	7.86 ± 0.08	0.16 ± 0.05	5.20 ± 0.25	27.08 ± 0.18
T1 (500 ppm)	7.62 ± 0.09	0.16 ± 0.05	5.05 ± 0.28	27.24 ± 0.22
T2 (1000 ppm)	7.50 ± 0.07	0.16 ± 0.05	5.10 ± 0.26	27.13 ± 0.19
T3 (1500 ppm)	7.42 ± 0.06	0.16 ± 0.05	5.08 ± 0.27	27.13 ± 0.21

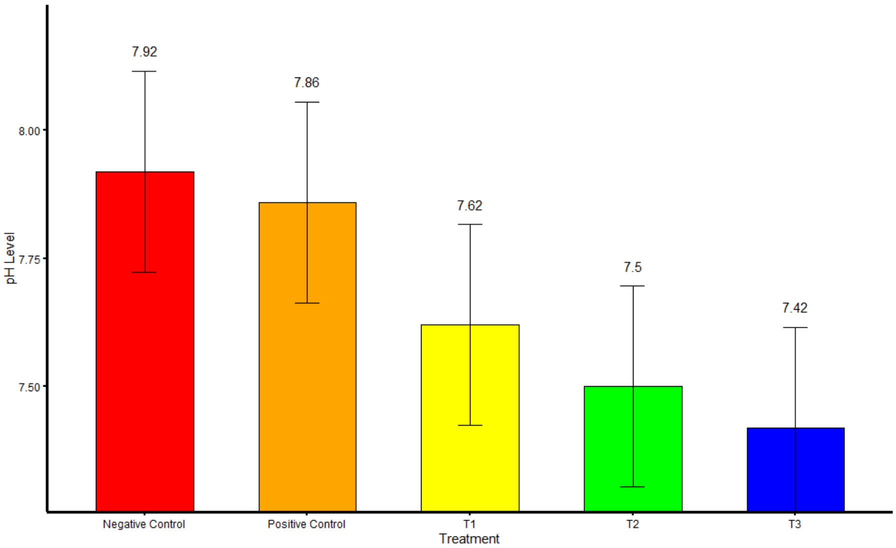

Figure 1. Changes in pH Across Experimental Groups Over Time
Error bars represent standard deviation. Differences were analyzed descriptively.

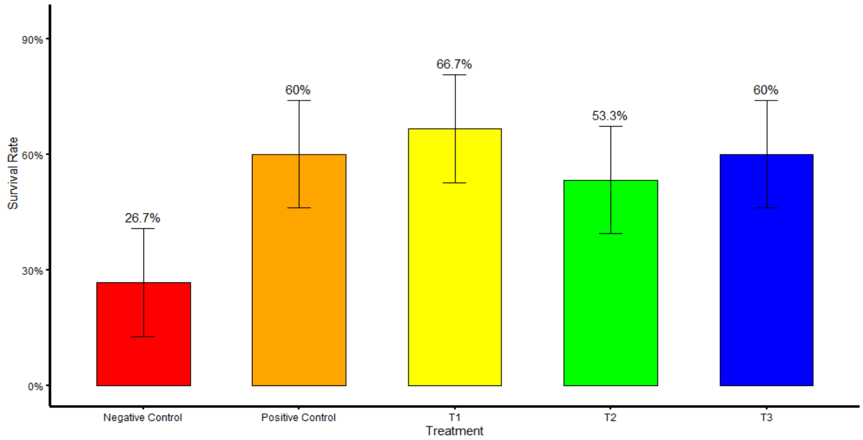

Figure 2. Survival Rate (%) of *P. reticulata* Across Treatments
Error bars represent standard deviation (n=15 fish per group). Chi-square test revealed a significant association between treatment and survival (χ^2, $p < 0.05$).

With the increasing concentration of the *T. catappa* leaf extract, the pH was observed to decrease, but within the optimal range. The pH level reduction may be attributed to the presence of phenolic compounds and tannins in *T. catappa*, which are known to acidify aquatic environments (Hung *et al.,* 2022; Wang *et al.,* 2024). According to a study by Widyastuti *et al.* (2021), the compounds extracted from the *T. catappa* leaf tea produced an acidic solution, demonstrating that the *T. catappa* leaves can lower the pH level when soaked in water.

A phytochemical study confirmed that *T. catappa* leaves contain phenolics, flavonoids, tannins, and other metabolites. The presence of tannins supports the mechanism by which the leaves lower pH when soaked in water (Chyau *et al.,* 2005).

The survival rate of *P. reticulata* under different extract concentrations

The study's results showed that fish survival was higher in all treatment groups relative to the negative control, which had the highest mortality. The 1000 ppm concentration consistently yielded the highest survival rates of 65% or the lowest mortality rate of 35%, indicating improved environmental conditions (Figure 2).

Improved survival rates among treated groups further support the beneficial effects of the lyophilized *T. catappa* (Talisay) leaf extract. The Talisay leaf extract enhances fish well-being and overall health through maintaining stable aquarium water conditions and reducing stressors, and the concentration of 1000 ppm (T2) provided the most balanced outcome among the treatment groups, exhibiting effective algal suppression without excessive alteration in water chemistry. The survival rate further supports the suitability of the extract as a water conditioning agent, wherein the treatment groups have significantly lower mortality compared to the negative control, suggesting a protective effect. Previous reports support this claim, indicating that *T. catappa* enhances and improves the fish's immune response, stress tolerance, and overall well-being (Wang *et al*., 2024). Consistent with previous studies, *T. catappa* extract improved survival observed in fish, demonstrating its beneficial effects on fish health. Nugroho *et al.* (2016) reported that the extract significantly increased the survival rate of *Betta sp.,* improved blood profile, and maintained favorable water quality. Similarly, Ramos *et al.* (2020) found that *T. catappa* significantly increased the survival of Amazon leaf fish larvae exposed to handling stress, suggesting that its protective effects improve stress tolerance during culture.

T. catappa leaf extract also improved survival and disease resistance in African catfish by enhancing immune competence and overall fish health (Orisasona *et al*., 2025). Furthermore, *T. catappa* leaves improved water quality in fish culture, which enhanced fish survival due to the favorable environmental conditions it creates (Gallano & Visca, 2022).

The anti-algal activity of the extract through algal growth

Visual assessment of algal growth

Visible green coloration, an indication of algal presence, was consistently observed in the negative control, positive control, and T1 (500 ppm) tanks during the 30-day experimental period, but no visible algal growth was observed in T2 (1000 ppm) and T3 (1500 ppm) (Figure 3).

At Day 0, all aquaria were initially clear, exhibiting uniform basal conditions. By Day 9, the negative control displayed early algal presence and progressed to a dense bloom on Day 21. On the other hand, a delayed onset of algal presence was observed in the Positive control, which progressed to a denser bloom by day 27. T1 (500 ppm) also showed delayed onset but less intense growth, wherein algal presence was first observed by day 21, without transitioning into a dense bloom. These findings indicate that lyophilized *Terminalia catappa* leaf extract can delay and suppress algal proliferation in a concentration-dependent manner. Even though it did not completely inhibit algal growth, T1 still showed reduced algal bloom intensity relative to the negative control, which suggest a partial algistatic activity.

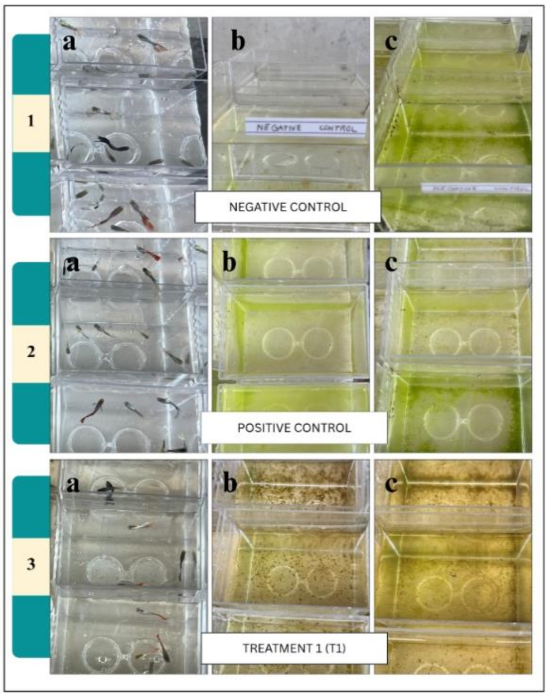

Figure 3. Algal Growth in tanks for Negative Control (1), Positive Control (2), and T1 (500 ppm) (3). *a1-3 (condition of the tanks at Day 1); b1-3 (initial appearance of green algal growth and coloration) and c1-3 (less algal growth to a denser one)*

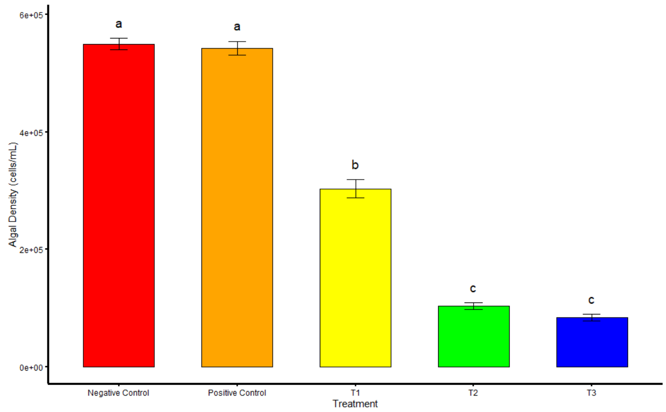

Figure 4. Algal Density (cells/mL) Across Treatment Groups
Bars represent mean values; error bars indicate standard deviation (n = 3). One-way ANOVA showed significant differences among groups (F(4,10) = 1448, p < 0.001). Different letters above bars indicate statistically significant differences among treatments based on the Tukey HSD post hoc test (p < 0.05).

Higher concentrations of the *T. catappa* leaf extract in T1 (1000 ppm) and T2 (1500 ppm) displayed no visible algal growth or green coloration, which is consistent with the quantitative reductions reported below (Figure 4). Tannins and other phenolic compounds found in *T. catappa* leaf is responsible for interfering with algal cell membranes and photosynthetic processes (Giang *et al.,* 2023; Ramanan *et al*., 2024; Wang *et al*., 2024). Reports from recent studies can further support this, where plant-derived polyphenols can inhibit chlorophyll synthesis and can disrupt the microalgae's cellular homeostasis, which leads to reduced biomass accumulation (Kumar *et al*., 2022; Del Mondo *et al*., 2022).

With the findings presented in this study, both the Aqua Care solution and the lyophilized *Terminalia catappa* leaf extract delayed algal proliferation in water compared with the negative control, and the extract concentration at ≥ 1000 ppm served the most effective algal suppression, which aligned with the statistical outcomes (Figure 4), supporting its application in aquaculture as a natural algicide.

Algal Density

There was a marked reduction in algal density observed in all treatment groups compared to the controls. One-way ANOVA followed by Tukey's post hoc test revealed significant differences in algal density among groups ($p < 0.05$), while the control groups showed no significant differences from each other, indicated by the same letter "a" on top of the bars. T1 (500 ppm) exhibited significant algal density reduction, marked by the different and separate letter "b", as compared to both controls. On the other hand, T2 and T3, treated with higher Talsiay concentration (1000 and 1500 ppm) respectively, were grouped together as "c", demonstrating that both were significantly different from the other treatments, which showed the lowest significant algal densities. This implied greater inhibitory effects (Figure 4).

These findings suggest that the extract contains compounds capable of inhibiting algal growth, thereby limiting excessive biomass accumulation.

This results in the stabilization of dissolved oxygen levels, as reduced algal respiration and decomposition lower or reduce oxygen demand within the system. The anti-algal effect of the Talisay extract was strongly supported by both the visual observations and quantitative analysis. Higher concentrations (1000 and 1500 ppm), represented by T2 and T3, respectively, significantly reduced algal density ($p < 0.001$), proving its inhibitory effect. This showed similar results reported in recent studies highlighting *T. catappa's* bioactive compounds as effective natural algicides (Ramanan *et al.,* 2024).

Efficacy Comparison of the Aqua Care Solution and Lyophilized *T. catappa* Leaf Extract

The positive control, treated with Aqua Care, demonstrated good performance in increasing dissolved oxygen, while lyophilized *Terminalia catappa* leaf extracts presented by T1, T2, and T3 exhibited comparable overall water conditioning efficacy in maintaining biologically suitable conditions for the *Poecilia reticulata* (Guppy). Both showed effective pH level stabilization and prevented oxygen depletion, which is a critical sign of water quality deterioration. The treatment groups' survival rates were comparable to those of the positive control, indicating that *T. catappa* leaf extract provided adequate environmental conditions to support fish health and well-being. The extract also reduced algal density significantly compared to both the control groups, demonstrating its potential to regulate algal growth.

These findings suggest that while Aqua Care improves physicochemical water parameters, specifically oxygen availability, *T. catappa* leaf extract offers a more sustainable alternative by promoting water stability, suppressing excessive algal growth, and enhancing fish survival. These observed differences are consistent with the intended functions of each treatment. Aqua Care intends to remove chlorine and neutralize chloramine, buffer pH, detoxify metals, improve water quality and reduce fish stress based on its manufacturer label, providing immediate improvement in water chemistry. As noted by Wesoly (2026), water conditioners are important during aquarium setup and water changes because it neutralizes harmful substances in tap water, reducing the risk of chemical stress on fish and supporting beneficial nitrifying bacteria.

In contrast, *T.catappa* leaf extract acts through natural bioactive compounds, like tannins and flavonoids, which are known to possess antimicrobial, antioxidant, and stress-reducing properties (Nugro *et al.,* 2016). These compounds have also been reported to reduce water turbidity, suppress microbial and algal growth, and enhance overall aquatic ecosystem productivity (Nozaleda, 2017; Nozaleda, 2019; Giang *et al.,* 2023; Pau *et al.,* 2022). However, there is limited evidence that currently demonstrates its ability to neutralize free chlorine or chloramine, indicating that its potential as a natural dechlorinating agent remains a vital area for future investigation. Overall, *T. catappa* leaf extract supports long-term water quality and fish health through its natural bioactive mechanisms, whereas Aqua Care provides rapid chemical water conditioning.

Determination of *T. catappa* leaf extract optimal concentration

Among the tested *Terminalia catappa* leaf extract concentration, T2 (1000 ppm) emerged as the most optimal level which provides a balance in water quality stability and effective algal growth suppression, and exhibited improved survival of the *Poecilia reticulata* without inducing excessive pH decrease, in line with the study's objective of identifying a practical and safe dosage for aquarium application, promoting environmentally friendly and sustainable alternative to commercial water conditioners that can help improve fish health and well-being.

Seeking an optimal concentration of an extract for aquarium fish is species-specific. Nugroho *et al.* (2016) found that concentrations of 375 ppm and above improve the blood profile and survival of *Betta sp.*, while Hashim *et al.* (2020) reported that high concentrations of the extract could be toxic to some cyprinid fish, indicating that the optimal concentration of *T. catappa* extract for aquarium use remains species-specific and application-dependent.

Previous studies have demonstrated that *T.catappa* leaves and their extracts possess antimicrobial, antioxidant, and immunostimulatory properties that improve fish health, survival, disease resistance and water quality in aquaculture (Wang *et al.,* 2024). Likewise, phytotherapeutic applications of *T. catappa* have primarily focused on preventing diseases, reducing stress, and enhancing fish welfare using fresh leaves, or conventional leaf extracts (Allesi *et al.,* 2019). Despite these documented benefits, published studies have largely emphasized the biological effects of *T. catappa* rather than the development of a standardized formulation for integrated water management. Limited studies have investigated the application of lyophilized *T. catappa* leaf extract as dual function water conditioner and algicide in Guppy (*Poecilia reticulata*) culture. Thus, this study addresses this gap by evaluating lyophilized (freeze-dried) *T. catappa* leaf extract based on the study's main objectives, to evaluate its efficacy as a natural water conditioner and an algicide.

This study contributes to science by providing evidence on the potential application of lyophilized *T. catappa* leaf extract as a natural water conditioner and algicide for Guppies (*P. reticulata*). While *T. catappa* has been widely recognized for its antimicrobial and water quality-enhancing properties (Chansue, 2011; Nair & Chanda, 2008), limited studies have evaluated its use in a lyophilized manner for dual functions in ornamental fish culture. The findings of this study may expand current knowledge on plant-based water treatment strategies and provide a scientific basis for the development of sustainable, eco-friendly alternatives to conventional chemical water conditioners.

Additionally, this study contributes to society by providing practical implications for aquarium management benefiting fish hobbyists and ornamental fish breeders, aquaculture practitioners, and manufacturers of aquatic care products. Through the use of plant-derived water conditioners, a safer, environmentally sustainable, and potentially more cost-effective approach to water quality management can be achieved, which is effective in controlling algal growth and may reduce reliance on synthetic chemical products for water conditioning. This study supports the broader goal of promoting sustainable ornamental fish culture and encouraging the utilization of renewable botanical resources in aquatic environmental management (Sung & Abol-Munafi, 2020).

In line with the United Nations Sustainable Development Goals, this study supports SDG 6 (Clean Water and Sanitation) by improving water quality management, SDG 12 (Responsible Consumption and Production) through the utilization of biodegradable and natural materials, and SDG 14 (Life Below Water) by minimizing ecological risks associated with chemical pollutants in aquatic systems, providing protection and safety to aquatic organisms.

CONCLUSION

Through all the findings presented, this study confirmed that lyophilized *Terminalia catappa* leaf extract can serve as a viable natural alternative to synthetic aquarium conditioners. The extract provides a sustainable and eco-friendly approach to aquarium management by enhancing fish survival, limiting algal proliferation, and improving overall water quality parameters and condition. The concentration of 1000 ppm was identified as the most optimal concentration, which effectively supports survival and well-being of the Guppy or *P. reticulata* and improves water quality by effectively suppressing algal growth without inducing a decrease in pH level, offering a balance between efficacy and safety of the fish, making way for its application in ornamental aquaculture. Through all the findings presented and the literature cited in this study, future investigations may evaluate the long-term efficacy of the lyophilized *Terminalia catappa* leaf extract and its application under larger-scale and diverse aquaculture conditions, optimal application rates, and its effect on beneficial aquatic microorganisms.

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AUTHOR CONTRIBUTION STATEMENT

All authors in this study gave significant contributions in completing this manuscript. ALMG designed the research study, analyzed the data, wrote the original draft of the manuscript, and conducted the experiment. LCC, OAA, and MRSB contributed to the revisions, review, and editing, providing invaluable insights to shape this study well. Overall, the authors contributed significantly to the work reported in this manuscript, the design and conception of the study, and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors of this study have no personal interests or conflicts of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tool was used in the design, data collection and data analysis of this study. All of the content drawn, the interpretation of results, or formation of scientific conclusions presented here were critically developed, analyzed, reviewed, verified, and approved entirely by the authors.

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