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Evaluation of Antibacterial Activity of Calamansi (*Citrus microcarpa*) Peel Extract

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ABSTRACT

This study investigated the antibacterial properties of calamansi (*Citrus microcarpa*) peel extract (CPE) at varying concentrations using the disk diffusion method. The primary objective was to evaluate its effectiveness in inhibiting bacterial growth in pond water used for tilapia (*Oreochromis sp.*) culture. Five treatments were applied, including a control (0%), pure calamansi peel extract (100%), and three diluted concentrations (0.5%, 1%, and 2%). Water samples from the pond, containing natural populations of heterotrophic bacteria commonly present in freshwater aquaculture systems, were used for bacterial inoculation. The antibacterial activity was assessed by measuring the inhibition zones around filter paper disks soaked in the extract. Results demonstrated that the pure CPE exhibited the highest antibacterial activity, with consistent inhibition zones across all trials. In contrast, the lower concentrations produced inconsistent and minimal inhibition effects. The control treatment showed no bacterial inhibition, confirming the absence of external antibacterial influences. The findings suggest that while pure CPE as the potential to serve as a natural antibacterial agent, the diluted concentrations are not effective for bacterial control in aquaculture systems. Further research is recommended to identify the active bioactive compounds, optimize the extract's formulation, and evaluate its long-term effects on aquatic microbial communities. By repurposing agricultural waste such as calamansi peels, this study contributes to SDG 12 (Responsible Consumption and Production) and SDG 14 (Life Below Water) through promoting sustainable aquaculture practices. The antibacterial potential of CPE also supports SDG 3 (Good Health and Well-being) by reducing reliance on synthetic antibiotics and mitigating the spread of antimicrobial resistance. Moreover, the use of natural antimicrobials in aquatic systems aligns with SDG 6 (Clean Water and Sanitation), helping ensure healthier aquatic environments.

Keywords : Calamansi Peel Extract, Antibacterial Activity, Disk Diffusion Method, Aquaculture, Natural Antibiotics

1 INTRODUCTION

Disease outbreaks remain one of the most significant challenges in aquaculture, resulting in substantial financial losses. Among these, bacterial infections are considered the primary culprits behind mass mortalities and epizootics in cultured aquatic species (Meyer, 1991). While fungal pathogens also contribute to production losses, bacterial diseases remain the most dominant threat. In the Philippine setting, the frequent use of antibiotics such as oxytetracyline, erythromycin, and florfenicol to control these infections is well documented (Tahiluddin & Terzi, 2021). However, the misuse and overuse of these antibiotics have raised concerns, particularly the alarming increase in antimicrobial resistance (AMR). This phenomenon not only limits treatment efficacy but also poses broader risks to food security and public health (Kraemer et al., 2019).

As the global population continues to grow, aquaculture plays a vital role in providing a safe, reliable, and affordable food supply (FAO, 2020). However, the increasing prevalence of antibiotic-resistant strains threatens not only the sustainability of aquaculture but also human well-being, undermining SDG 3 (Good Health and Well-being). The emergence of resistant bacteria reduces the effectiveness of medical treatments, leading to higher mortality rates from infections that were once easily treatable.

While the misuse of antibiotics in clinical and agricultural settings remains a major driver of AMR, natural processes such as horizontal gene transfer and genetic mutations also contribute to its persistence. Aquaculture systems, often characterized by dense microbial populations, serve as hotspots for the exchange of genetic material, including antibiotic resistance genes. The coexistence of antibiotics, probiotics, prebiotics, and other treatments in these systems further facilitates this genetic exchange (Watts et al., 2017). In the Philippine context, studies have highlighted how intensive aquaculture operations contribute to environmental degradation and the proliferation of resistant bacterial strains due to improper antibiotic use and waste discharge (Tahiluddin et al., 2025). Understanding the sources and transmission pathways of antimicrobial resistance within aquaculture systems is crucial to safeguarding both human and environmental health, particularly in tropical archipelagic nations like

the Philippines where aquaculture is a major food and economic resource (Watts et al., 2017)—closely aligning with SDG 14 (Life Below Water).

In the search for alternative solutions to combat bacterial infections, natural plant-based compounds have gained considerable attention. Fruits belonging to the Rutaceae family, including calamansi (*Citrus microcarpa*), are known for their rich phytochemical content. These bioactive compounds exhibit antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, and anti-arthritic properties (Azman et al., 2019). Notably, the non-edible parts of calamansi, particularly the peel, contain significant concentrations of flavanones and polymethoxylated flavones, compounds with potent antibacterial properties (Viuda-Martos et al., 2008; Kamaludin et al., 2015).

Given its abundance and potential bioactivity, calamansi peel extract presents a promising, eco-friendly alternative to conventional antibiotics. In the Philippines, large quantities of calamansi peels are discarded as agro-industrial waste, particularly from juice vendors, house-holds, and food processors (Zalameda et al., 2014; Husni et al., 2020). These organic residues often end up in landfills or open dumpsites, contributing to solid waste management challenges and missed opportunities for resource recovery (Gonzaga-Torino et al., 2025). Tapping into this waste stream for antibacterial applications supports SDG 12 (Responsible Consumption and Production) by promoting circular economy strategies and reducing organic waste.

Calamansi peels are rich in bioactive compounds such as flavonoids, essential oils, and phenolics with known antimicrobial properties (Azman et al., 2019; Ali et al., 2017). By valorizing these peels for their antibacterial potential, this study not only explores a sustainable method of bacterial control in aquaculture but also contributes to SDG 6 (Clean Water and Sanitation) through the reduction of synthetic inputs in fishponds. This study aims to evaluate the antibacterial properties of calamansi peel extract in aquaculture water, offering insights into its potential application in managing bacterial infections and promoting sustainable aquaculture practices in support of global and national sustainability targets.

2 METHODOLOGY

2.1 Location and duration of the Study

The study was carried out at the College of Fisheries Laboratory, located in Katipunan, Roseller T. Lim, Zamboanga Sibugay, Philippines. The experimental work, including sample preparation, antibacterial assays, and data collection, was conducted over a two-month period from November to December 2023.

2.2 Factor and Treatment

This study employed a single experimental factor: the concentration of calamansi (*C. microcarpa*) peel extract (CPE) incorporated into nutrient agar plates inoculated with 100 µL of tilapia pond water. The primary objective was to assess the antibacterial activity of varying CPE concentrations against bacteria naturally present in the aquaculture environment.

Five treatment levels were formulated to represent different concentrations of CPE, ranging from no extract (control) to pure extract (undiluted). The treatments are summarized in Table 1.

Table 1. Calamansi peel extract (CPE) treatments and their corresponding concentrations

Treatment	Calamansi Extract Concentration	Description
1	0% (Control)	No CPE (untreated)
2	100%	Pure CPE without dilution
3	0.5%	5 mL CPE in 1L distilled water
4	1%	10 mL CPE in 1L distilled water
5	2%	20 mL CPE in 1L distilled water

These treatments allowed for the evaluation of CPE efficacy across a gradient of concentrations, facilitating the identification of the most effective dosage for inhibiting bacterial growth in aquaculture settings.

2.3 Preparation of the calamansi peel extract (CPE)

Fresh *C. microcarpa* (calamansi) fruits were procured from a local market in Zamboanga Sibugay, Philippines. Upon acquisition, the fruits were thoroughly rinsed with tap water to eliminate surface contaminants and allowed to air dry at room temperature. Once dry, the peels were manually separated from the fruit and subjected to an additional air-drying process for approximately one hour to reduce residual surface moisture.

The air-dried peels were then chopped into uniform small pieces using a sanitized knife or food processor. To facilitate the release of bioactive compounds and essential oils, the chopped peels were ground using an electric grinder. The resulting pulp was enclosed in a clean cheesecloth and subjected to mechanical pressing to extract the juice. Sufficient pressure was applied to ensure maximum yield.

The expressed liquid was subsequently filtered using a coffee filter to remove residual solid particles, yielding a clear, CPE. The extract was divided into three portions (5 mL, 10 mL, and 20 mL) to prepare the experimental treatment solutions corresponding to 0.5%, 1%, and 2% concentrations, respectively, when diluted in 1 liter of distilled water. An undiluted portion was also reserved for the 100% treatment.

All extracts were transferred into sterilized, labeled amber bottles to minimize light-induced degradation and stored under refrigeration (4 °C) until used in antibacterial assays.

2.4 Culture Media Preparation

Nutrient agar (NA) was prepared by dissolving 11.2 g of commercially available nutrient agar powder in 400 mL of distilled water using a sterile reagent bottle. The solution was gently swirled and heated to a boil to ensure complete dissolution of the medium. Once fully dissolved, the medium was subjected to sterilization in an autoclave at 121 °C (15 psi) for 15 mins to eliminate potential contaminants.

After autoclaving, the sterilized nutrient agar was allowed to cool to approximately 45 °C–50 °C to prevent condensation and preserve the medium's integrity. The molten agar was then aseptically poured into pre-sterilized Petri dishes in an improvised laminar airflow cabinet or near an alcohol lamp to maintain a sterile environment. Each Petri dish was filled with 15–20 mL of agar.

Once solidified, the agar plates were sealed with parafilm or laboratory tape to prevent contamination and placed in a clean, covered glass container. The plates were stored at room temperature for 24 hrs prior to use to confirm sterility and ensure no microbial growth was present before inoculation with bacterial samples.

2.5 Antibacterial test using Disk Diffusion Method

The antibacterial activity of CPE was assessed using the standardized disk diffusion method as described by Hudzicki (2009). Water samples were obtained from a tilapia *(Oreochromis* sp.) culture pond and diluted in a 1:10 ratio (1 mL pond water to 9 mL sterile distilled water). From this, 100 µL of the diluted sample was aseptically spread

onto the surface of pre-prepared NA plates.

A total of five (5) treatments were tested, each replicated twice (2 plates per treatment). Each plate contained six (6) sterile filter paper disks (Whatman No. 1, 6 mm diameter), resulting in 12 disks per treatment.

Filter disks were soaked in their respective CPE solutions for approximately 30 mins to ensure sufficient absorption. After drying briefly under sterile conditions, the disks were carefully placed onto the inoculated agar plates using sterile forceps—6 disks per plate, evenly spaced to avoid overlapping inhibition zones.

All inoculated plates were incubated at room temperature for 24 hrs to allow for bacterial growth and interaction with the treatments. Following incubation, the zones of inhibition—clear areas surrounding the disks indicating bacterial growth suppression—were measured in millimeters using a transparent plastic ruler. The average diameter per treatment was calculated and used as a quantitative indicator of antibacterial efficacy.

2.6 Data Collection

Data collection was conducted 24 hrs after the incubation period. The antibacterial activity of the CPE was evaluated by measuring the zone of inhibition—the clear area surrounding each filter paper disk where bacterial growth was visibly suppressed.

The diameter of the inhibition zones was measured in millimeters (mm) using a transparent plastic ruler for consistency and accuracy. Each petri dish contained six disks per replicate, and the inhibition zones for all disks within each treatment were measured. The average inhibition zone per replicate was then computed and recorded for further analysis.

These measurements were used to assess and compare the antibacterial efficacy of the different extract concentrations. The collected data were subsequently subjected to statistical analysis to determine whether significant differences existed among treatments.

3 Results And Discussion

The antibacterial activity of fresh CPE was evaluated using the disk diffusion assay. The results are presented in Table 2 showing the average inhibition zone diameters (in mm) at different concentrations across three trials. The inhibition zone indicates the extract's antibacterial effectiveness, with larger zones representing stronger antibacterial activity (Figure 1).

The results indicate that the antibacterial activity of fresh calamansi peel extract varied depending on its concentration. Across all trials, the pure extract (100% CPE) consistently exhibited the highest inhibition zones, with diameters ranging from 12 mm to 16 mm. This demonstrates the potent antibacterial properties of the undiluted extract, likely due to the higher concentration of bioactive compounds such as flavonoids, phenolics, and essential oils (Namsa et al., 2011).

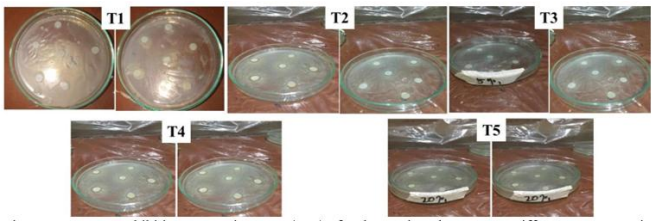

Figure 1. Average Inhibition Zone Diameters (mm) of Calamansi Peel Extract at Different Concentrations

At a concentration of 0.5% CPE (5 mL in 1 L pond water), inhibition zones were inconsistent, ranging from 0 mm to 8 mm across replicates and trials. Similarly, the 1% CPE (10 mL in 1 L pond water) exhibited moderate antibacterial activity, with inhibition zones between 0 mm and 10 mm, though variability was observed between replicates. On the other hand, the 2% CPE (20 mL in 1 L pond water) showed mixed results, with inhibition zones ranging from 0 mm to 8 mm. This inconsistent response suggests that while calamansi peel extract has antibacterial potential, the activity at lower concentrations may be less reliable and dependent on factors such as bacterial strain susceptibility and environmental conditions.

The control group, which did not contain any calamansi peel extract, exhibited no inhibition zones in all trials. This confirms the absence of any natural antibacterial interference in the experimental setup.

The fresh CPE was assessed to determine its antibacterial capability in reducing bacterial growth in pond water. The water samples used in this study were collected from a tilapia (*Oreochromis* sp.) culture pond, which typically contains a diverse population of naturally occurring heterotrophic marine bacteria involved in nutrient cycling and organic matter decomposition. Although no specific bacterial species were isolated or identified in this study, the presence of heterotrophic marine bacteria in pond environments is well-documented and serves as a realistic target for evaluating antimicrobial interventions. The results from the trials indicate that the extract significantly inhibited bacterial growth, suggesting its potential application in aquaculture. These findings support the use of CPE as a natural antibacterial agent, offering an eco-friendly alternative to synthetic antibiotics. This could be particularly beneficial for fish farmers aiming to reduce antibiotic use and minimize the risk of antibiotic resistance.

Previous studies have also demonstrated the antimicrobial properties of citrus peels. For example, Dollah et al. (2019) found that *C. microcarpa* and *C. aurantiifolia* were effective in turbidity removal and exhibited antibacterial properties. Additionally, Li et al. (2024) highlighted the antioxidant, anti-inflammatory, and anticancer properties of citrus peel essential oils, further supporting the therapeutic potential of calamansi peel extract.

Furthermore, Duremdes et al. (2022) demonstrated the synergistic antibacterial effect of calamansi and banana peels against *Escherichia coli* using a disk diffusion assay. Their findings align with the present study, reinforcing the potential of CPE as an effective antibacterial agent. The antibacterial activity of calamansi peel is primarily attributed to its high content of bioactive compounds, such as flavonoids (e.g., hesperidin), limonoids, and essential oils like D-limonene. These compounds disrupt bacterial cell walls and membranes, interfere with enzyme function, and cause leakage of intracellular components, ultimately leading to bacterial cell death (Fisher & Phillips, 2008; Husni et al., 2020). This mechanism supports the observed inhibition zones in the present study, indicating that CPE has a promising potential for natural disease management in aquaculture systems.

Overall, the results provide a strong foundation for further exploration of CPE in aquaculture and other applications. Additional research involving larger sample sizes, more bacterial strains, and detailed phytochemical analysis could enhance the understanding of its antibacterial mechanisms and optimize its practical use.

4 CONCLUSION

Based on the findings of this study, it can be concluded that pure calamansi peel extract (100%) demonstrated the most significant antibacterial effect, exhibiting the largest inhibition zones across all trials. This suggests that calamansi peel extract has the potential to serve as a natural antibacterial agent. However, the lower concentrations (0.5%, 1%, and 2%) showed minimal and inconsistent inhibition effects, indicating limited antibacterial properties at these levels. The absence of significant inhibition zones at these concentrations implies that diluted calamansi peel extract may not be effective in reducing bacterial growth in cultured water. While the extract's antibacterial potential is evident at higher concentrations, further research is necessary to explore its practical application, optimize treatment methods, and assess its long-term impact in aquaculture systems. Comprehensive evaluation is essential to validate the extract's safety, efficacy, and suitability as an alternative to synthetic antibiotics.

REFERENCES

Ali, J., Das, B., & Saikia, T. (2017). Antimicrobial activity of lemon peel (Citrus limon) extract. *International Journal of Current Pharmaceutical Research, 9*(4), 79. https://doi.org/10.22159/ijcpr.2017v9i4.20962

Azman, N. F. I. N., Azlan, A., Khoo, H. E., & Razman, M. R. (2019). Antioxidant properties of fresh and frozen peels of citrus species. Current Research in Nutrition and Food Science, 7(2), 331–339. https://doi.org/10.12944/crnfsj.7.2.03

Dollah, Z., Abdullah, A. R. C., Hashim, N. M., Albar, A., Badrealam, S., & Zaki, Z. Z. M. (2019). Citrus fruit peel waste as a source of natural coagulant for water turbidity removal. Journal of Physics Conference Series, 1349(1), 012011. https://doi.org/10.1088/1742-6596/1349/1/012011

Duremdes, K., Revil, T., Iral, J., & Bingcang, A. (2022). An in vitro study on the synergistic effect of the Musa acuminata and Citrofortunella microcarpa peel extract against Escherichia coli. 8ISC Proceedings: Allied Health, 103–111. https://ejournal.unklab.ac.id/index.php/8ISCAH/article/view/620

FAO. (2020). *The State of World Fisheries and Aquaculture 2020: Sustainability in action.* Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en

Fisher, K., & Phillips, C. A. (2008). Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends in Food Science & Technology*, 19(3), 156–164. https://doi.org/10.1016/j.tifs.2007.11.006

Gonzaga-Torino, F. M., Candole, K. P., Bajalla, M. L. B., Catienza, F. A., Anuevo, G. M., Yürüten Özdemir, K., Apines-Amar, M. J. S., Camarin, M.-A. M., Fagutao, F. F., Choresca Jr., C. H., & Pedroso, F. L. (2025). Fermented calamansi (Citrus microcarpa) waste as a functional feed additive: Nutritional, antioxidant, and anti-nutritional insights for tilapia (*Oreocrhromis* sp.). International Journal of Aquatic Biology, 13(3), 19–33. https://doi.org/10.22034/ijab.v13i3.2472

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. Retrieved from https://asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol

Husni, E., Ismed, F., & Afriyandi, D. (2020). Standardization Study of Simplicia and Extract of Calamondin (*Citrus microcarpa* Bunge) Peel, Quantification of Hesperidin and Antibacterial Assay. Pharmacognosy Journal. https://doi.org/10.5530/pj.2020.12.111

Kamaludin, R., Ibrahim, N. H., Zakaria, Z., Dachyar, A., & Ahmad, N. H. (2015). Antibacterial properties of Limau Kasturi (Citrus microcarpa) peel extract. Advances in Environmental Biology, 9(19 S4), 5+. Retrieved from https://link.gale.com/apps/doc/A440635601/AONE?u=anon~79f975b8&sigo

Kraemer, S. A., Ramachandran, A., & Perron, G. G. (2019). Antibiotic pollution in the environment: From microbial ecology to public policy. Microorganisms, 7(6), 180. https://doi.org/10.3390/microorganisms7060180

Li, Y., Li, W., Ye, Z., Ji, C., & Zhou, Z. (2024). Antioxidant, anti-inflammatory, and anticancer activities of five citrus peel essential oils. Antioxidants, 13(12), 1562. https://doi.org/10.3390/antiox13121562

Meyer, F. W. (1991). Aquaculture disease and health management. Journal of Animal Science, 69(10), 4201–4208. https://doi.org/10.2527/1991.69104201x

Namsa, N. D., et al. (2011). Antibacterial activity of *Citrus microcarpa* peel extract. Journal of Medicinal Plants Research, 5(17), 4026–4034.

Tahiluddin, A. B., & Terzi, E. (2021). A review of reported bacterial diseases and antibiotic use in tilapia culture in the Philippines. Acta Natura et Scientia, 2(2), 141-147.

Tahiluddin, A. B., Bornales, J. C., Limbaro, G. R. A., Paudac, M. A. U., Amarille, R. K., Sirad, N. R., Kabirun, M. C., Ujing, R. A., Gonzaga-Torino, F. M., Sabdani, M. H., Bacla-an, R. E., Hairal, M. A. S., Magcanta-Mortos, M. L. M., and Esguerra, J. P. (2025). Environmental impacts of aquaculture in the Philippines. *Israeli Journal of Aquaculture–Bamidgeh, 77*(2), 51–81.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2008). Functional and antimicrobial properties of citrus and their by-products. *Critical Reviews in Food Science and Nutrition, 48*(4), 417–437. https://doi.org/10.1080/10408390701279745

Watts, J. E. M., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks, and solutions. Marine Drugs, 15(6), 158. https://doi.org/10.3390/md15060158

Zalameda, E. C., Falco, M. E. M., Esteban, L. R., & Florendo, N. E. C. (2014). Livelihood Technology Series 11: Calamansi Processing. Department of Science and Technology- Industrial Technology Development Institute, DOST Compound, General Santos Avenue, Bicutan, Taguig City, Metro Manila, Philippines.