

**FROM PEEL TO PURITY: PINEAPPLE (*Ananas comosus*) WASTE
EXTRACT AS A NATURAL DEPURATION OF BACTERIAL
CONTAMINANTS FOR GREEN MUSSEL (*Mytella strigata*)**

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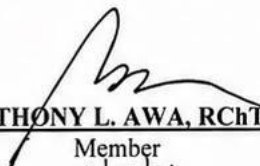
DEPARTMENT OF NATURAL SCIENCES

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ABSTRACT

Green mussels (*Mytella strigata*) are widely consumed in the Philippines. However, their filter-feeding nature renders them susceptible to the accumulation of harmful bacteria, including *Vibrio* spp., which are known to cause foodborne illnesses. The conventional chlorine-based depuration method employed to sanitize these mussels poses environmental concerns and incurs additional costs. Concurrently, pineapple (*Ananas comosus*) peel, a commonly discarded agricultural waste, contains natural bioactive compounds with potential antibacterial properties. This study investigated the efficacy of pineapple peel extract as a natural alternative for reducing *Vibrio* spp. in green mussels. Pineapple peels were subjected to ethanol extraction, and the resulting extract was prepared at varying concentrations for experimental testing. Fresh green mussels from Tanguib City were treated with artificial seawater (control), chlorine solution, and pineapple peel extract for 48 hours. Bacterial testing using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar showed that selected pineapple peel extract treatments completely removed detectable *Vibrio* colonies after 48 hours. Statistical analysis revealed significant differences among treatment groups at 24 hours ($H = 16.27$, $p = 0.0124$) and 48 hours ($H = 29.51$, $p < 0.0001$). The results also demonstrated that bacterial counts decreased significantly over time in all pineapple-treated groups, including the 12.5%, 25%, and 50% concentrations. Further analysis indicated that pineapple peel extract performed as effectively as chlorine, with no significant difference observed between them. Overall, this study demonstrates that pineapple peel extract is a promising, low-cost, and eco-friendly alternative for reducing harmful bacteria in green mussels (*Mytella strigata*) and could be effectively utilized as a natural depuration agent.

Keywords: *agricultural by-products, bacterial reduction, coastal food safety, ethanolic extract, natural depuration agent*

DEDICATION

This research study is wholeheartedly dedicated to God Almighty for His endless guidance, wisdom, strength, and countless blessings throughout the completion of this study. It is also dedicated to our cherished parents and families for their unwavering love, sacrifices, understanding, encouragement, and constant prayers throughout this journey.

This work is likewise dedicated to our mentors, especially Yunalyn L. Villanted, MSc. Bio, Ph.D.; Ma'am Nelfa D. Canini, Ph.D.; and Karl Maxel O. Lao, RMT, MSc. MLS, for their invaluable guidance, patience, encouragement, and expertise, which greatly contributed to the accomplishment of this research.

The researchers also dedicate this study to the College of Medical Technology, Misamis University for providing the academic environment and opportunities that made this endeavor possible.

Lastly, this work is dedicated to all seekers of knowledge whose passion for learning and discovery continues to inspire the pursuit of research and academic excellence.

The Researchers

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INTRODUCTION

Background of the study

Green mussels, *Mytella strigata*, play an important role in aquaculture by providing a significant source of food, livelihood, and economic support for many coastal communities in the Philippines. However, because mussels are filter-feeding organisms, they are highly susceptible to accumulating microbial contaminants present in surrounding waters, posing serious concerns to food safety and public health (Nuñal et al., 2023). Pathogenic microorganisms commonly associated with contaminated mussels include *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Salmonella* spp., *Escherichia coli*, and Norovirus (Afreen & Bağdatli, 2021). Consumption of raw or undercooked contaminated shellfish may result in gastrointestinal infections, septicemia, and other severe foodborne illnesses in humans (Estrada, 2023).

The susceptibility of mussels to microbial contamination is explained by the Bioaccumulation Theory, which states that filter-feeding aquatic organisms continuously accumulate microorganisms and contaminants from their surrounding environment through feeding and respiration processes. Because mussels filter large volumes of water daily, pathogenic bacteria present in polluted coastal waters may concentrate within their tissues at levels higher than those found in the surrounding environment. This biological process makes mussels effective bioindicators of marine pollution but also increases the risk of transmitting foodborne pathogens to consumers.

To minimize microbial contamination, depuration has been widely practiced in shellfish processing. Depuration involves placing live mussels in clean and controlled seawater systems to allow the natural elimination of contaminants from their tissues (Brander et al., 2025). This process is anchored on the Self-Purification Theory, which explains that bivalves can naturally expel contaminants through continuous filtration and metabolic activity when transferred to uncontaminated water. Effective depuration improves shellfish quality, enhances consumer safety, and ensures compliance with food sanitation standards. In addition, it reduces the risk of foodborne disease outbreaks and strengthens consumer confidence in aquaculture products, thereby supporting the sustainability of coastal economies dependent on shellfish farming (Campbell et al., 2022).

Despite its effectiveness, conventional depuration systems present several economic and environmental challenges, particularly for small-scale aquaculture operators. Many depuration facilities rely on chemical disinfectants such as chlorine and ultraviolet (UV) treatment systems, which require costly infrastructure, continuous energy consumption, and technical maintenance (Curran et al., 2019). Prolonged exposure to chemical disinfectants may also alter the natural microbiota, flavor, and texture of mussels, potentially affecting product quality (Lingham et al., 2016). Moreover, the operation of depuration systems requires constant aeration, water circulation, and temperature regulation, contributing to increased electricity consumption and operational costs (Jayabal & Sivanraju, 2025). These limitations highlight the need for sustainable, affordable, and environmentally friendly alternatives that can effectively reduce microbial contamination without relying heavily on synthetic chemicals.

The search for plant-based antimicrobial alternatives is supported by the Phytochemical Theory, which explains that plants synthesize secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds as natural defense mechanisms against microorganisms. These bioactive compounds possess antimicrobial properties that can inhibit bacterial growth through membrane disruption, enzyme inhibition, protein denaturation, and interference with microbial metabolic pathways. The theory provides the scientific basis for investigating agricultural plant wastes as potential natural antibacterial agents.

Simultaneously, agricultural waste management remains a major environmental concern in the Philippines. Large volumes of biodegradable agricultural by-products are generated annually from agro-industrial activities, and improper disposal contributes to environmental pollution, greenhouse gas emissions, and ecological degradation (DENR, 2025). Among these agricultural commodities, pineapple (*Ananas comosus*) is one of the country's major fruit crops, particularly in Northern Mindanao, where pineapple processing industries generate substantial amounts of peel, pulp, and core wastes each year (Philippine Statistics Authority, 2023). Although often discarded, pineapple peels are rich in bioactive compounds such as bromelain, phenolic acids, flavonoids, tannins, and saponins, which possess reported antibacterial and antioxidant properties (Mehraj et al., 2024).

The utilization of pineapple peel waste in this study is further supported by the Waste Valorization Concept, which emphasizes converting agricultural wastes into valuable and functional products instead of treating them as disposable materials. Waste valorization promotes sustainability by reducing environmental burdens while

maximizing the economic and practical value of agricultural by-products. In the context of this study, transforming pineapple peel waste into a natural depuration agent aligns with sustainable aquaculture and circular economy principles.

Recent studies have explored the antimicrobial potential of plant-based extracts and agricultural wastes against foodborne pathogens. Pal and Mahant (2025) reported that *Moringa oleifera* leaf extract exhibited strong antibacterial activity against *Vibrio* spp. and *E. coli* in aquaculture systems. Similarly, Hussain et al. (2022) demonstrated that *Carica papaya* peel extract inhibited the growth of *Salmonella typhimurium* and *Staphylococcus aureus*. These findings support the Natural Antimicrobial Theory, which proposes that naturally derived compounds from plants may serve as safer and environmentally sustainable alternatives to synthetic antimicrobial agents.

However, limited studies have investigated the use of pineapple peel waste extract as a natural depuration agent for shellfish, particularly against *Vibrio* spp. contamination in green mussels. Addressing this research gap may provide an innovative approach that integrates aquaculture food safety with agricultural waste valorization. Therefore, this study evaluated the antibacterial potential of pineapple (*Ananas comosus*) peel waste extract as a natural depuration agent against *Vibrio* spp. in green mussels (*Mytella strigata*). Specifically, the study aimed to determine its effectiveness in reducing bacterial load during depuration and to assess its potential as a sustainable alternative to conventional chemical disinfectants.

Objectives of the study

This study generally aimed to evaluate the potential of pineapple (*Ananas comosus*) peel waste extract as a natural depuration agent for green mussels (*Mytella strigata*). Specifically, the study sought to:

1. Determine the antibacterial activity of pineapple peel waste extract against *Vibrio* spp. in green mussels (*Mytella strigata*);
2. Assess the effectiveness of pineapple peel waste extract in reducing microbial load during the depuration process; and
3. Compare the depuration efficacy of pineapple peel waste extract with chlorine as a conventional depuration agent at varying concentrations (12.5%, 25%, and 50% v/v) over 24- and 48-hour treatment periods

Significance of the study

This study is significant because it explored a sustainable and eco-friendly approach to improving food safety in aquaculture through the utilization of pineapple (*Ananas comosus*) peel waste extract as a natural depuration agent for green mussels (*Mytella strigata*). By investigating the antibacterial potential of pineapple peel extract against *Vibrio* spp., the study contributed to the development of alternative depuration methods that may reduce dependence on conventional chemical disinfectants commonly used in shellfish processing.

The findings of the study may benefit mussel growers, particularly small-scale aquaculture producers, by providing a potentially cost-effective and locally available

depuration alternative that can help reduce operational expenses associated with chemical-based depuration systems. The study may also benefit consumers by contributing to safer mussel consumption through the reduction of microbial contamination and foodborne health risks associated with pathogenic bacteria.

In terms of environmental significance, the study promoted the practical utilization of agricultural waste materials, specifically pineapple peel residues, which are commonly discarded despite containing bioactive compounds with antimicrobial properties. The conversion of agricultural waste into a value-added product supports sustainable waste management practices and aligns with environmental conservation and circular economy principles.

Furthermore, the results of this study may serve as a reference for future researchers conducting studies on plant-based antimicrobial agents, natural depuration systems, and sustainable aquaculture technologies. The findings may likewise provide baseline information for policymakers, environmental agencies, and aquaculture stakeholders in developing long-term strategies related to food safety, agricultural waste utilization, and environmentally sustainable aquaculture practices.

Scope and Delimitations

This study focused on evaluating the potential of pineapple (*Ananas comosus*) peel waste extract as a natural depuration agent for green mussels (*Mytella strigata*). Specifically, the study assessed its antibacterial activity against *Vibrio* spp. and its effectiveness in reducing microbial load during the depuration process under controlled laboratory conditions. The study also compared the depuration efficacy of pineapple peel

extract with chlorine at varying concentrations (12.5%, 25%, and 50% v/v) over 24- and 48-hour treatment periods.

The microbiological analysis was limited only to bacterial contamination, particularly *Vibrio* spp., using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar as the selective medium. Viral, fungal, parasitic, and chemical contaminants were not included in the investigation. Only crude ethanolic extracts of pineapple peel were utilized in the experiment, and the specific bioactive compounds responsible for the observed antibacterial activity were neither isolated nor chemically characterized.

The study was conducted solely under laboratory-controlled conditions and did not extend to field validation in actual aquaculture or commercial depuration systems. Physicochemical parameters of the depuration water, such as dissolved oxygen, salinity, pH, and temperature, were not extensively monitored throughout the experimental period. Furthermore, the investigation focused only on short-term depuration outcomes and immediate post-treatment bacterial reduction. Long-term storage stability, shelf-life, sensory quality, and possible bacterial regrowth after depuration were not evaluated.

In addition, the study was performed within a single sampling period and did not account for seasonal variations in microbial contamination levels or possible variations in the phytochemical composition of pineapple peel due to cultivar differences, maturity stage, or processing conditions. Potential variations in extract potency among preparation batches were likewise not extensively examined.

MATERIALS AND METHODS

Research Design

This study employed a quantitative experimental research design to evaluate the antibacterial effectiveness of pineapple (*Ananas comosus*) peel waste extract as a natural depuration agent for green mussels (*Mytella strigata*). Specifically, the study investigated the ability of pineapple peel extract to reduce *Vibrio* spp. contamination in green mussels during the depuration process under controlled laboratory conditions.

The experiment utilized both control and experimental treatment groups. The control groups consisted of untreated seawater and chlorine-treated depuration systems, while the experimental groups involved varying concentrations of pineapple peel extract (12.5%, 25%, and 50% v/v). Green mussels were subjected to depuration treatments for 24 and 48 hours to assess time-dependent antibacterial effects.

Microbiological analysis focused on the enumeration of *Vibrio* spp. using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar as the selective culture medium. Bacterial counts were expressed as colony-forming units per gram (CFU/g) and were determined before and after treatment application. The resulting data were quantitatively analyzed to compare microbial load reduction among treatment groups and evaluate the depuration efficacy of pineapple peel extract relative to conventional chlorine treatment.

Research Setting

Fresh pineapple (*Ananas comosus*) peels used in this study were obtained from a local fruit vendor to ensure the availability and freshness of the plant material used for

extraction. Fresh green mussels (*Mytella strigata*) were collected from coastal areas in Tangub City, specifically near Brgy. Maloro, to ensure sample freshness and quality prior to experimentation (Figure 1).

The preparation of pineapple peel extract, depuration procedures, microbiological analyses, bacterial culturing, and data collection were conducted at the laboratory facilities of Misamis University under controlled laboratory conditions. The laboratory provided the necessary equipment, culture media, and environmental conditions required for extraction, microbial testing, and experimental analysis.

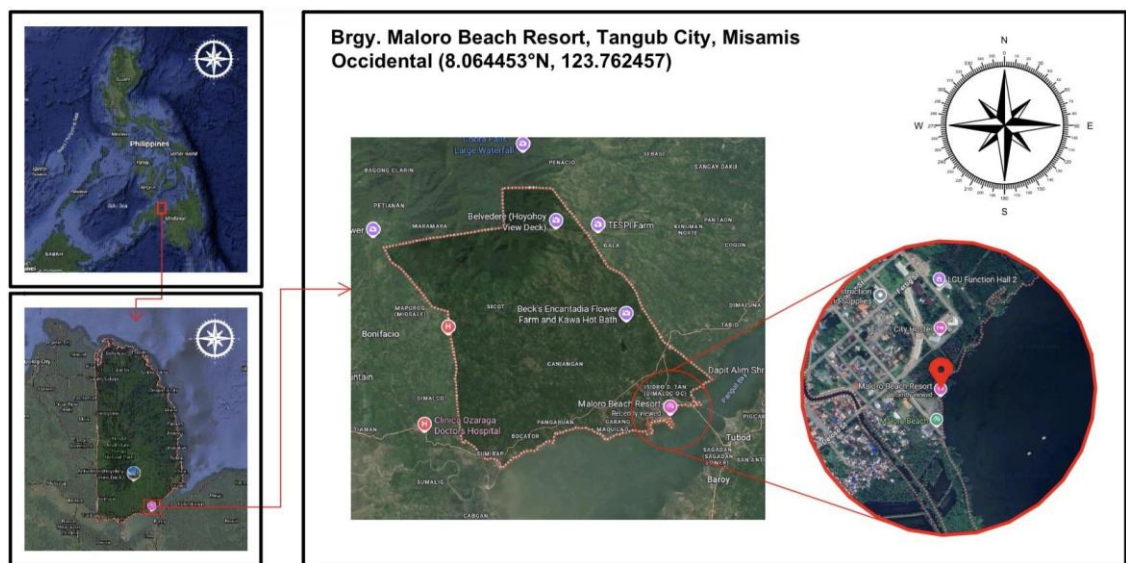


Figure 1. Brgy. Maloro Beach Resort, Tangub City, Misamis Occidental (8.064453°N, 123.762457)

Sample Species Identification

Prior to sample collection and experimentation, species identification of the shellfish samples was conducted to ensure the accuracy and validity of the study (Figure 2). Green mussel samples collected from coastal areas near Brgy. Maloro Beach Resort ,

Tangub City, were submitted to the Bureau of Fisheries and Aquatic Resources for morphological identification and verification of the species as *Mytella strigata*.



Figure 2. Specimen for morphological analysis of *Mytella strigata* (Hanley, 1843)

Conceptual Framework

Figure 3 presents the schematic diagram of the study, which illustrated the stepwise methodology employed for the extraction of bioactive compounds from pineapple (*Ananas comosus*) peels and their subsequent application as a natural depuration agent for green mussels (*Mytella strigata*).

As shown in the diagram, the process commenced with the collection, washing, air-drying, pulverization, and weighing (500 g) of fresh pineapple peels. The powdered material was then subjected to maceration in 70% ethanol at room temperature for 72 hours with regular agitation. The resulting filtrate, obtained through decantation using muslin cloth and filtration with Whatman No. 1 filter paper, was subsequently concentrated using a rotary evaporator. The dried or freeze-dried extract was then stored at 4 °C and later reconstituted into stock solutions using sterile distilled water.

Concurrently, fresh green mussels were collected from Brgy. Maloro Beach Resort and transported in insulated coolers with wet ice. Upon arrival, the mussels were rinsed with artificial seawater and randomly assigned to various treatment groups, including control groups (A and B) and experimental groups exposed to 12.5%, 25%, and 50% v/v concentrations of the pineapple peel extract, alongside chlorine and artificial seawater controls.

Following the depuration period (24 and 48 hours), both mussel tissues and water samples were collected at designated time points (0, 24, and 48 hours). These samples were then subjected to serial 10-fold dilutions in sterile buffered peptone water and spread-plated onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar, a selective medium for *Vibrio spp.* After incubation, colony counting and presumptive identification were performed to enumerate bacterial populations. The bacterial counts obtained from both pre- and post-depuration tissue samples, as well as from water samples, were subsequently analyzed to evaluate the antimicrobial efficacy and overall depuration performance of the pineapple peel extract treatments.

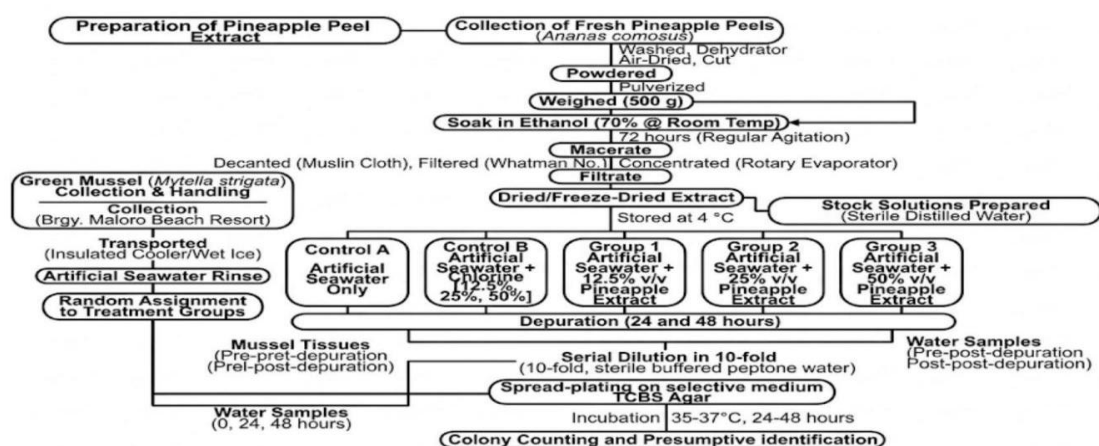


Figure 3. Schematic diagram of the study

Preparation of Pineapple Peel Extract

Fresh pineapple (*Ananas comosus*) peels were collected, washed thoroughly to remove dirt and residues, and cut into small pieces. The cut peels were then placed in a dehydrator set at 50°C for approximately 24 hours until they became completely dry and brittle. The use of a dehydrator at this temperature was chosen over sun drying because sun drying exposes the material to fluctuating temperatures, UV radiation, and potential microbial contamination, which can degrade bioactive compounds such as bromelain and phenolic acids. Controlled drying at 50°C has been reported to preserve enzymatic activity and phenolic content more effectively than sun drying or higher temperatures (Tan et al., 2020; Othman et al., 2021).

Once dried, the peels were pulverized into a fine powder using a clean grinder. Approximately 500 grams of the pineapple peel powder were weighed and macerated in 2,000 mL of 70% ethanol at room temperature for 72 hours, with regular manual agitation applied throughout the period. This method was adapted from the extraction protocol described by Sandamali et al. (2024). After maceration, the mixture was decanted through muslin cloth and then filtered using Whatman No. 1 filter paper, following standard procedures in ethanol-based extraction. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator and further dried to obtain the crude extract. The dried extract was stored at 4°C until use. The concentration step using the rotary evaporator was carried out at Mindanao State University – Iligan Institute of Technology (MSU IIT). This process yielded a total of 212.5 mL of crude extract. Stock solutions were prepared by dissolving the dried extract in sterile distilled water to achieve working concentrations of 12.5%, 25%, and 50% v/v, following the concentration ranges used in

previous antimicrobial studies (Purnamasari, 2024; Sharma et al., 2022). Since each of the three experimental groups required 70.8 mL of extract, the total volume needed for one complete depuration run was 212.4 mL, which closely matched the available yield. This alignment was consistent with the observations of Muhamad et al. (2020), who noted that researchers often had to adjust treatment volumes based on actual extraction yields when working with plant materials. The process is presented in Figure 4.



Figure 4. Preparation of Pineapple Peel Extract

Green mussel (*Mytella strigata*) Collection and Handling

Green mussels (*Mytella strigata*) were divided into seven treatment groups, each housed in a separate tank containing 15 mussels. The groups consisted of one negative control using artificial seawater only, three positive control groups using chlorine at different dilution levels, and three experimental groups using pineapple peel extract at different concentrations. Research by Swastawati et al. (2021) demonstrated that pineapple solution, a source of citric acid, can be used to effectively reduce heavy metal levels in green mussels (Swastawati et al., 2021).



*Figure 5. Green mussel (*Mytella strigata*) Collection and Handling*

Depuration Treatments and Water Sampling

Green mussels (*Mytella strigata*) were divided into seven treatment groups, each housed in a separate tank containing 15 mussels. The groups consisted of one negative control using artificial seawater only, three positive control groups using chlorine at different dilution levels, and three experimental groups using pineapple peel extract at different dilution levels.

Preparation of Artificial Seawater

The artificial seawater used in all tanks was prepared by dissolving commercially available table salt in distilled water. Food-grade table salt was selected because sodium chloride was the primary salt required to achieve the appropriate salinity for marine bivalves. Distilled water served as the solvent to eliminate any microorganisms or organic matter that could confound the experimental results. Artificial seawater prepared from distilled water and sodium chloride contains negligible bacterial loads and is suitable for controlled microbiological experiments. *Mytella strigata* maintains optimal filtration and survival rates within this process of using artificial seawater. Using sterile or near sterile water in shellfish depuration studies prevents external microbial interference.

Dilution Design and Stock Solution

The six treatment groups followed the same dilution principle. A fixed volume of 70.8 mL of the active agent was used in every treatment tank. This fixed volume was determined by the total amount of pineapple peel crude extract obtained after rotary evaporation, which was 212.5 mL. Dividing this total equally among the three experimental groups yielded 70.8 mL per group. The chlorine treatments were then matched to this same volume to maintain consistency across all groups. Keeping the active agent volume constant while adjusting the water volume allows for clearer dose-response comparisons.

The treatment labels of 12.5%, 25%, and 50% did not refer to the final chemical concentration in the tank. Rather, these percentages indicated the proportion of the total tank volume that came from the stock solution. A 12.5% treatment meant that 12.5% of

the liquid in the tank was stock solution and 87.5% was artificial seawater. A 25% treatment meant 25% stock solution and 75% seawater. A 50% treatment meant equal parts stock solution and seawater.

Chlorine Stock and Working Concentrations

The chlorine stock solution was an 8% sodium hypochlorite solution of 1 gallon , a commercially available lab-grade formulation. The actual sodium hypochlorite concentration in each treatment tank was calculated by multiplying the stock concentration of 8% by the treatment proportion. For the 12.5% treatment, the final chlorine concentration was 8% multiplied by 0.125, which equaled 1.0%. For the 25% treatment, it was 8% multiplied by 0.25, which equaled 2.0%. For the 50% treatment, it was 8% multiplied by 0.50, which equaled 4.0%.

Volume Calculations

The volume of artificial seawater required for each treatment was calculated by first determining the total final volume and then subtracting the fixed stock volume. The total final volume was obtained by dividing the stock volume of 70.8 mL by the desired treatment proportion.

For the 50% treatment, the total final volume was 70.8 mL divided by 0.50, which equaled 141.6 mL. Subtracting the 70.8 mL of stock solution left 70.8 mL of artificial seawater. For the 25% treatment, the total final volume was 70.8 mL divided by 0.25, which equaled 283.2 mL. Subtracting 70.8 mL left 212.4 mL of artificial seawater, rounded to 212 mL. For the 12.5% treatment, the total final volume was 70.8 mL divided

by 0.125, which equaled 566.4 mL. Subtracting 70.8 mL left 495.6 mL of artificial seawater, rounded to 495 mL.

Concentration Selection

The three treatment proportions were chosen to create a dose-response gradient. The 50% upper limit was set because preliminary observations indicated that higher proportions of pineapple peel extract increased viscosity and altered pH beyond acceptable levels for mussel survival. The 12.5% lower limit represented the minimum proportion expected to produce measurable antibacterial effects within the 48-hour depuration window. The 25% midpoint provided an intermediate dose for assessing whether bacterial reduction scaled proportionally with concentration.

Although the total tank volumes differed across treatments, each group received the same absolute volume of active agent. The smallest total volume of 141.6 mL, used in the 50% treatments, was confirmed to be sufficient for complete submersion of all 15 mussels.

Depuration Conditions and Water Sampling

Depuration was carried out over 24 and 48 hours. Aseptic water samples were collected from each tank at the 24- and 48-hour marks only. No samples were taken at 0 hours. At the start of the experiment, each tank contained freshly prepared artificial seawater made from distilled water and table salt, which was essentially sterile. Sampling at 0 hours would have yielded zero counts across all tanks and provided no comparative value. The purpose of water sampling was to track whether bacteria purged from the

mussels accumulated in the surrounding water. Water sampling was conducted to track bacteria purged from mussels into the surrounding water. Depuration typically results in gradual bacterial release from bivalves, with changes in waterborne counts becoming detectable over the course of the process (Venkatesh et al., 2026).

Limiting sampling to two time points also preserved the tank water volume and minimized disturbance to the mussels. Limiting sampling also preserved tank water volume and minimized disturbance to the mussels to avoid stressing the animals and altering treatment concentrations.

The absence of 0-hour water data did not compromise the study, as the primary endpoint was bacterial load in mussel tissues measured at 0, 24, and 48 hours. The absence of 0-hour water data did not compromise the study, as the primary endpoint was bacterial load in mussel tissues measured at 0, 24, and 48 hours. Tissue bacterial counts remained the definitive measure of depuration efficacy, with water samples providing supplementary context.



Figure 6. Depuration Treatments

Sample Preparation and Microbiological Enumeration

Green mussel (*Mytella strigata*) samples from each treatment tank were collected aseptically at 0, 24, and 48 hours for microbiological analysis. Mussel tissues were aseptically removed, homogenized in sterile buffered peptone water at a 1:9 (w/v) ratio, and subjected to ten-fold serial dilutions. From the appropriate dilutions, 0.1 mL aliquots were spread-plated onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar for the selective isolation of *Vibrio spp.* Plates were incubated at 35–37°C for 24 hours following established microbiological procedures (Purnamasari, 2024; Sharma et al., 2022).

To monitor bacterial levels in the depuration medium, water samples were aseptically collected from each tank at 24 and 48 hours. Each 1 mL water sample underwent ten-fold serial dilution, and 0.1 mL aliquots from selected dilutions were spread-plated onto TCBS agar to obtain isolated colonies for enumeration. This procedure enabled the assessment of bacterial reduction in both mussel tissues and depuration water throughout the treatment period.

After incubation, visible colonies characteristic of *Vibrio spp.* were counted, and bacterial load was expressed as colony-forming units per gram (CFU/g) of mussel tissue using the formula (1):

$$CFU/g = \frac{(Average\ colony\ count \times dilution\ factor \times 10)}{weight\ of\ sample\ (g)}$$

Percent reduction in bacterial load will be calculated using this formula (2):

$$\%Reduction = \frac{(CFU_{pre} - CFU_{post})}{CFU_{pre}} \times 100$$

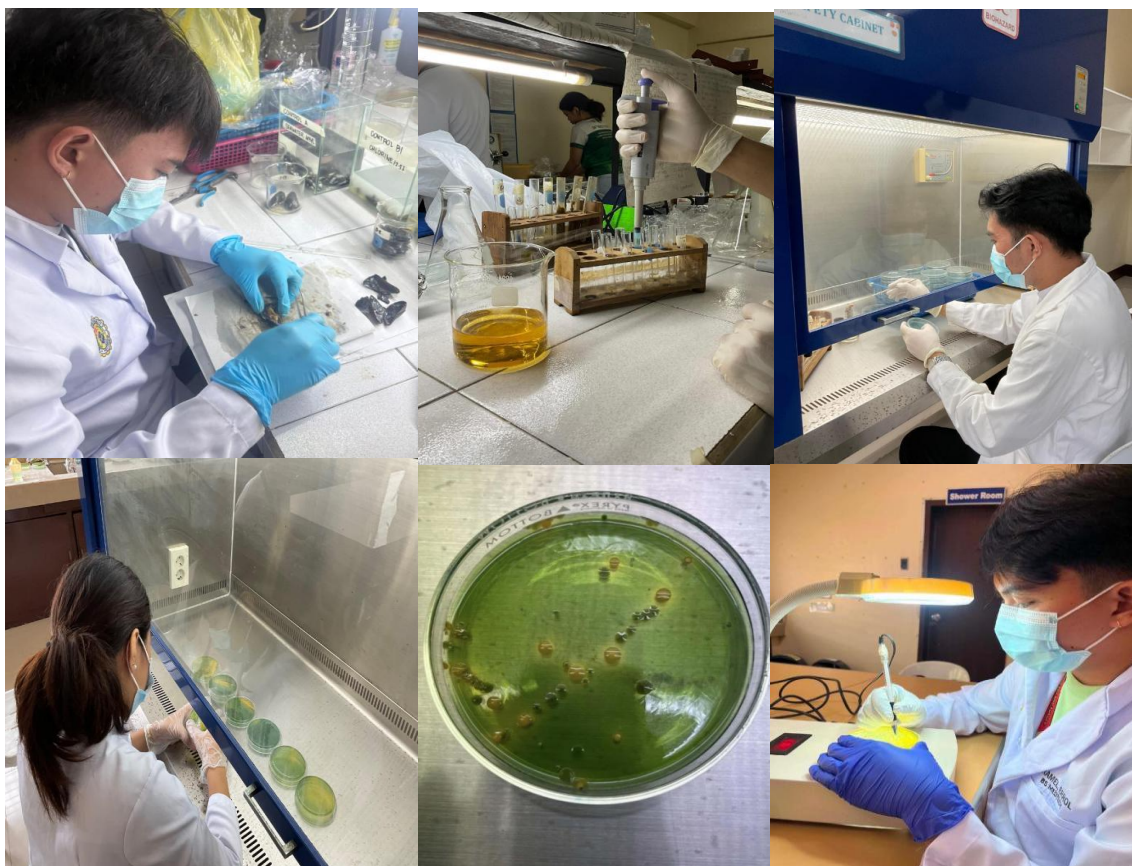


Figure 7. Sample Preparation and Microbiological Enumeration

Presumptive Identification of Target Bacteria

For the selective enumeration of presumptive *Vibrio spp.*, Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar was used as the selective medium. Following incubation, all colonies exhibiting typical *Vibrio* morphology were counted. Colony appearance on TCBS agar, such as green (sucrose negative) and yellow (sucrose positive) colonies, was noted to support presumptive identification and to distinguish *Vibrio* species from any other microorganisms that may have been present in the samples (Oliver, 2015).

On TCBS agar, the color of the colonies helped differentiate between species of *Vibrio* based on their ability to ferment sucrose. This differentiation was possible because TCBS agar contained sucrose as a fermentable carbohydrate and bromothymol blue as a pH indicator. When a bacterium fermented sucrose, it produced acid byproducts that lowered the pH of the medium surrounding the colony, causing the bromothymol blue indicator to shift from its original green-blue color to yellow. Bacteria that did not ferment sucrose left the medium unchanged, so their colonies retained a green or blue-green appearance. TCBS agar is highly effective for isolating *Vibrio* species from seafood samples, and distinct colony colors allow for rapid presumptive differentiation in the laboratory (Letchumanan et al., 2014). However, those authors also cautioned that some bacterial species other than *Vibrio* could grow on the medium, so colony color should be interpreted together with other findings.

Yellow colonies on TCBS agar indicated sucrose fermentation and were presumptively identified as *Vibrio cholerae*, *Vibrio alginolyticus*, or *Vibrio fluvialis*. Yellow colonies on TCBS agar correspond to sucrose-fermenting species (Letchumanan et al., 2014). Among these yellow colony formers, *Vibrio cholerae* was the most medically significant because it caused cholera, a severe diarrheal disease marked by profuse watery diarrhea that could lead to rapid dehydration and death if left untreated (Sahra, 2018). *Vibrio cholerae* consistently produces yellow colonies on TCBS agar due to its ability to ferment sucrose (Sahra, 2018). *Vibrio alginolyticus* is commonly associated with wound infections and external ear infections, especially in people exposed to warm seawater (Oliver, 2005). *Vibrio alginolyticus* among the most frequently detected sucrose-fermenting species, producing characteristic large yellow colonies on

TCBS agar (Ismail et al., 2024). *Vibrio fluvialis* is linked to gastroenteritis, causing symptoms such as watery diarrhea, abdominal cramps, and vomiting (Usta et al., 2018). *Vibrio fluvialis* is increasingly recognized as a cause of diarrheal illness in coastal communities and can be presumptively identified by its yellow colony morphology on TCBS agar (Ramamurthy et al., 2014).

Green colonies on TCBS agar indicated that the bacteria did not ferment sucrose, leaving the medium unchanged (Nigro et al., 2011). These green colonies were presumptively identified as *Vibrio parahaemolyticus* or *Vibrio vulnificus* (Nigro et al., 2011). The symptoms included watery diarrhea, nausea, vomiting, and abdominal cramps that typically appeared within 24 hours of consuming contaminated shellfish (Ritchie et al., 2012).

Vibrio vulnificus was considered one of the most dangerous *Vibrio* species because it could cause severe wound infections and primary septicemia (Xu et al., 2023). Leng et al. (2022) reported that infections could progress rapidly, with a fatality rate exceeding 50 percent in septicemic cases, particularly among individuals with underlying health conditions such as liver disease, diabetes, or weakened immune systems (Heng et al., 2017).

Further studies supported the continued use of TCBS agar for monitoring *Vibrio* contamination in aquaculture products. Elbashir et al. (2020) evaluated methods for detecting *Vibrio* species in seafood and concluded that TCBS agar remained a reliable and practical selective medium for routine screening in food safety laboratories (Yeung & Thorsen, 2016). The authors noted that the ability to differentiate between sucrose-

fermenting and non-fermenting colonies provided valuable preliminary information about the species present. Roy et al. (2021) studied the microbiological quality of bivalves collected from different harvesting areas and used TCBS agar to isolate and presumptively identify *Vibrio* species based on colony color (Song et al., 2020). The study reported that green colonies were the most frequently observed in their samples, suggesting a higher prevalence of *Vibrio parahaemolyticus* or *Vibrio vulnificus* compared to sucrose-fermenting species. A recent review emphasized the risks associated with *Vibrio* species in seafood and the importance of effective depuration methods to reduce bacterial loads before the products reached consumers ((BIOHAZ) et al., 2024).

The health risks linked to *Vibrio* contamination in shellfish were well documented in recent literature(Brauge et al., 2023). Froelich and Daines (2020) reviewed the ecology and pathogenicity of *Vibrio* species in estuarine and marine environments and reported that filter-feeding bivalves concentrated these bacteria from the surrounding water, making them a significant vehicle for human infection(Destoumieux-Garzón et al., 2020). The study noted that climate change and rising sea surface temperatures were contributing to the increased prevalence of *Vibrio spp.* in coastal waters, raising further concern for seafood safety. Brumfield et al. (2022) examined the environmental factors influencing *Vibriopopulations* in oysters and reported that temperature and salinity were key drivers of bacterial abundance(Quigg et al., 2025). The authors emphasized that depuration and post-harvest processing were critical control points for reducing the risk of illness.

By noting the colony color on TCBS agar, the study was able to make a reasonable presumptive identification of the types of *Vibrio* present in the green mussel samples. This

approach provided useful information about the potential health risks associated with the bacterial contamination without requiring additional biochemical or molecular testing.

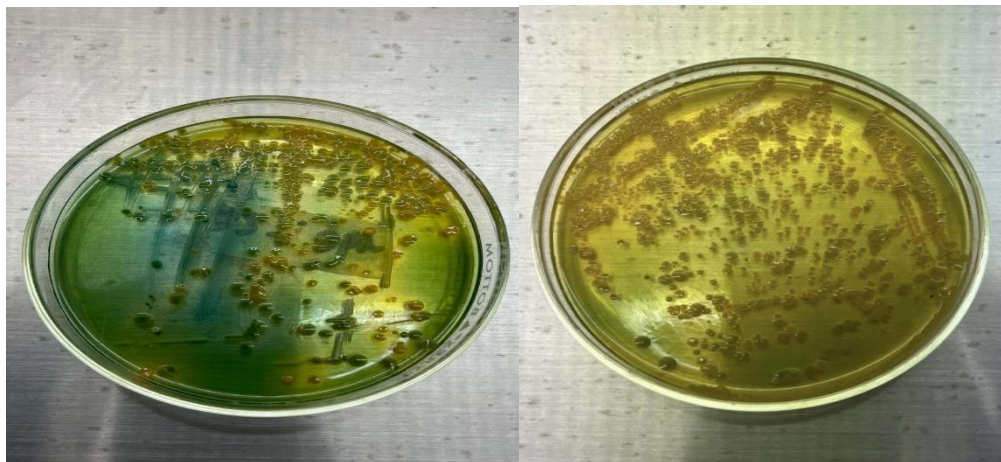


Figure 8. Presumptive Identification of Target Bacteria

Data Analysis

The bacterial counts obtained from the green mussel samples were expressed as colony-forming units per gram (CFU/g) and were transformed into logarithmic values ($\log_{10}$ CFU/g) prior to statistical analysis to minimize variability and improve data normalization. Descriptive statistics, including mean and standard deviation (SD), were computed for each treatment group and sampling interval (0, 24, and 48 hours).

Statistical Tests

The normality of the data distribution was assessed using the Shapiro–Wilk test. Since several datasets did not satisfy the assumption of normality, non-parametric statistical tests were employed for inferential analysis. Differences among treatment

groups at each sampling interval were analyzed using the Kruskal–Wallis test. When significant differences were detected, Dunn's post hoc test was performed to identify specific pairwise differences among treatments.

To determine changes in bacterial counts over time within each treatment group, the Friedman test was used as the non-parametric alternative to repeated measures ANOVA. Statistical significance for all analyses was set at $\alpha = 0.05$. All statistical analyses were performed using the Paleontological Statistics Software Package (PAST) version 4.03 and IBM SPSS Statistics version 29.

RESULTS AND DISCUSSION

Antibacterial Activity of Pineapple (*Ananas comosus*) Peel Extract Against *Vibrio* spp. in Green Mussels (*Mytella strigata*) During Depuration

At the beginning of the experiment (0 hr), all treatment groups exhibited relatively high bacterial counts ranging from 1.69 to 2.40 log₁₀ CFU/g, confirming the initial presence of *Vibrio* contamination in the *Mytella strigata* samples (Table 1). These baseline levels are consistent with findings from Nuñal et al. (2023), who reported similar *Vibrio* contamination levels in oysters and mussels along Philippine market supply chains (Nuñal et al., 2023). Recent studies (Kijewska et al., 2023) have also documented that *Vibrio* species are commonly isolated from marine bivalves in tropical environments. Scientific evidence (BIOHAZ et al., 2024) has further noted that *Vibrio parahaemolyticus* and *Vibrio vulnificus* are prevalent in seafood from warm waters, and It has been further added (Ramamurthy et al., 2014) that *Vibrio fluvialis* is an emerging pathogen in coastal regions.

Table 1. Mean *Vibrio* spp. Counts in Green Mussels During Depuration Using Chlorine and Pineapple Peel Extract Treatments

Treatment	0 hr (log₁₀ CFU/g)	24 hr (log₁₀ CFU/g)	48 hr (log₁₀ CFU/g)	% Reduction (0–48 hrs)	Interpretation
Control A	2.40	2.48	2.44	~0%	No reduction
Chlorine 12.5%	1.78	1.80	0.68	92.2%	Strong reduction
Chlorine 25%	2.02	1.95	0.00	100%	Complete elimination
Chlorine 50%	2.37	2.10	0.00	100%	Complete elimination
Pineapple 12.5%	1.69	1.25	0.00	100%	Complete elimination
Pineapple 25%	2.07	1.49	0.53	97.1%	Strong reduction
Pineapple 50%	2.37	0.87	0.00	100%	Complete elimination

*Legend: **No reduction** - less than 10% reduction (control group showed no significant bacterial decrease), **Strong reduction** - 90% to 99% reduction (substantial antibacterial activity but incomplete elimination), **Complete elimination** - 100% reduction (0.00 log₁₀ CFU/g, no detectable *Vibrio* colonies)*

The interpretation categories used were based on established antimicrobial efficacy classifications reported in previous studies (Table 1). Complete elimination of detectable bacterial colonies, defined as a 100% reduction or 0.00 log₁₀ CFU/g, indicates that the treatment concentration and exposure time were sufficient to fully inactivate the target bacteria (Mainous et al., 2011). Reductions between 90% and 99% are classified as "strong reduction," demonstrating substantial antibacterial activity even when complete elimination is not achieved (Fadel & El-Lamie, 2019). Reductions below 10% are considered "no significant reduction," which aligns with the control group interpretation in the present study. Ampofo & Clerk (2020) further noted that in aquaculture disinfection studies, complete elimination or 100% reduction was regarded as the desired outcome for

effective sanitization protocols, while strong reduction of at least 90% was still considered acceptable for practical applications.

In addition, the pineapple peel waste extract treatments produced notable antibacterial activity against *Vibrio spp.* in *Mytella strigata* (Table 1)(Taka et al., 2024). Pineapple 50% showed the most rapid reduction, decreasing sharply from 2.37 to 0.87 log₁₀ CFU/g within 24 hours (Table 1). After 48 hours, Pineapple 12.5% and Pineapple 50% achieved complete elimination of detectable *Vibrio* colonies, while Pineapple 25% reduced bacterial counts to 0.53 log₁₀ CFU/g, representing a 97.1% reduction (Table 1). These findings demonstrate that pineapple peel waste extract possesses strong antibacterial activity against *Vibrio spp.* in green mussels. Similar results have been reported(Mulia et al., 2023), showing that pineapple peel extract enhanced immunity and resistance to *Vibrio parahaemolyticus* in penaeid shrimp. Other studies found that pineapple peel ethanol extract demonstrated strong antibacterial activity against common fish and shellfish pathogens(Taka et al., 2024). A comprehensive qualitative analysis of the phytochemical profile of pineapple peel extract(Soni et al., 2025) identified numerous bioactive compounds responsible for its antimicrobial effects.

The observed antibacterial activity of pineapple peel waste extract against *Vibrio spp.* in *Mytella strigata* may be attributed to the combined action of bromelain, phenolic compounds, flavonoids, tannins, and organic acids naturally present in pineapple peel (Table 1). Li et al. (2021) reported that bromelain, a proteolytic enzyme, damages bacterial cell walls by breaking down glycoprotein components of the cell membrane, resulting in increased membrane permeability and eventual bacterial cell death(Mamo, 2019). Phenolic compounds such as ferulic acid, gallic acid, and catechin derivatives

inhibit bacterial growth by destabilizing cytoplasmic membranes and interfering with nucleic acid synthesis (Borges et al., 2012). Mehraj et al. (2024) comprehensively documented that pineapple peel contains multiple bioactive compounds with broad-spectrum antimicrobial properties, supporting its use as a natural antimicrobial agent against *Vibrio spp.* Hussain et al. (2022) emphasized that fruit peels represent a valuable source of bioactive natural products, turning agricultural waste into value-added products. Chen et al. (2021) also demonstrated dose-dependent antibacterial activity of phenolic extracts from fruit peels against foodborne pathogens, which aligns with the concentration-dependent effects observed in the present study.

The water sample data provided additional evidence regarding the antibacterial activity of pineapple peel waste extract against *Vibrio spp.* in the depuration system (Table 3). The control tanks remained heavily contaminated throughout the experiment, with bacterial counts too numerous to count (TNTC), indicating persistent *Vibrio* contamination in the depuration water (Table 3). In contrast, pineapple peel extract treatments substantially reduced bacterial counts in the water column. Pineapple 12.5% and Pineapple 50% achieved complete elimination of detectable *Vibrio* colonies by the end of the depuration period, and Pineapple 25% significantly reduced bacterial counts to 3.40 CFU (Table 3). These findings indicate that pineapple peel waste extract reduced *Vibrio* contamination not only within *Mytella strigata* tissues but also in the surrounding depuration water (Table 3).

Assessing the Effectiveness of Pineapple Peel Waste Extract in Reducing Microbial Load During the Depuration Process

The effectiveness of pineapple peel waste extract in reducing microbial load during depuration was assessed over 24 and 48 hours. The results showed that prolonged

exposure time enhanced bacterial reduction in *Mytella strigata*, with greater reductions observed after 48 hours than after 24 hours across all pineapple peel extract treatments (Table 1).

Table 2. Friedman Test (Time Effect Within Treatments)

Treatment	χ^2	df	p-value	Decision
Control A	1.00	2	0.607(A)	Fail to reject H_0
Chlorine 12.5%	7.89	2	0.019(B)	Reject H_0
Chlorine 25%	6.78	2	0.034(B)	Reject H_0
Chlorine 50%	8.32	2	0.016(B)	Reject H_0
Pineapple 12.5%	8.40	2	0.015(B)	Reject H_0
Pineapple 25%	9.58	2	0.008(B)	Reject H_0
Pineapple 50%	10.00	2	0.007(B)	Reject H_0

Legend: **A** = not significant at $p \geq 0.05$, **B** = significant at $p < 0.05$, χ^2 - Chi-square statistic (Friedman test), **df** - Degrees of freedom, **H_0** - null hypothesis (no significant change over time)

The Friedman test was used to determine whether significant changes in bacterial counts occurred over time within each treatment group (Table 2). The control group showed no statistically significant change across the three sampling periods ($\chi^2 = 1.00$, $df = 2$, $p = 0.607$), confirming that untreated seawater alone did not significantly reduce *Vibrio* populations in *Mytella strigata* during depuration (Table 2). Methodological considerations in depuration research must account for natural purging rates, which are often insufficient without antimicrobial intervention (Martinez-Albores et al., 2020).

In contrast, all pineapple peel extract treatments demonstrated statistically significant reductions over time (Table 2). Pineapple 12.5% ($\chi^2 = 8.40$, $p = 0.015$), Pineapple 25% ($\chi^2 = 9.58$, $p = 0.008$), and Pineapple 50% ($\chi^2 = 10.00$, $p = 0.007$) exhibited highly significant reductions over time (Table 2). Plant-derived compounds require sufficient contact time to achieve maximum bacterial reduction in bivalve species (Khameneh et al., 2019). Natural antimicrobials from plants often require extended

contact times compared to synthetic chemicals, but they offer the advantage of lower toxicity and environmental persistence(Haktaniyan & Bradley, 2022).

Table 3. Mean CFU Counts in Water Samples

Treatment	Mean 24 hrs CFU	Mean 48 hrs CFU	Interpretation
Control A (Seawater)	TNTC (>300 CFU)	TNTC (>300 CFU)	Highly contaminated
Chlorine 12.5%	62.75	4.75	Effective
Chlorine 25%	90.10	0.00	Complete elimination at 48 hrs
Chlorine 50%	126.60	0.00	Complete elimination at 48 hrs
Pineapple 12.5%	17.80	0.00	Effective
Pineapple 25%	31.20	3.40	Strong reduction
Pineapple 50%	7.40	0.00	Complete elimination

Legend: TNTC - Too Numerous To Count (>300 CFU per plate), Complete elimination - 0.00 CFU detected after 48 hours, Strong reduction - $\geq 90\%$ reduction but not complete elimination, Effective reduction - substantial decrease in bacterial counts but not complete elimination

The water sample interpretations used were supported by previous studies on depuration water quality (Table 3). Chinnadurai et al. (2022) explained that bacterial release from bivalves during depuration occurred gradually as filtration activity progressed, and water column contamination was a reliable indicator of purging efficiency. Prunte et al. (2020) observed that untreated depuration water remained heavily contaminated over time, which they classified as "highly contaminated" when bacterial counts exceeded 300 CFU per plate, a threshold that aligned with the TNTC designation in the present study. Martinez et al. (2021) reported that effective reduction in water column bacteria was achieved when counts dropped below 10 CFU after 48 hours, while strong reduction referred to decreases of at least 90% even when complete

elimination was not reached. Phuvasate & Su (2020) further noted that complete elimination of detectable colonies in depuration water, or 0.00 CFU, was considered the ideal outcome for preventing recontamination of shellfish tissues. Tamplin & Capers (2021) emphasized that monitoring water quality during depuration was essential, and reductions in waterborne bacterial counts directly correlated with the effectiveness of the antimicrobial treatment being tested.

Furthermore, the water sample data further supported the effectiveness of pineapple peel waste extract in reducing microbial load during the depuration process (Table 3). Pineapple 12.5% and Pineapple 50% achieved complete elimination of detectable *Vibrio* colonies in the water column by 48 hours, while Pineapple 25% reduced counts to 3.40 CFU (Table 3). This dual action of reducing microbial load in both mussel tissues and surrounding water enhances overall depuration efficiency. Chinnadurai et al. (2022) noted that the ability to reduce water column bacteria is a critical advantage in depuration systems, as it prevents expelled bacteria from being re-filtered by bivalves. For *Mytella strigata*, which continuously pumps water through its gills at high rates, maintaining clean depuration water is essential for effective microbial load reduction. Rizal et al. (2020) studied the effect of salinity on filtration rate and survival of green mussels, confirming that high filtration rates make them particularly susceptible to recontamination from dirty water.

The time-dependent nature of microbial load reduction aligned with the established understanding of how bivalve depuration works. As a filter feeder, *Mytella strigata* naturally purges contaminants from its tissues when placed in clean water, but this process requires sufficient time and favorable environmental conditions. The

dynamics of bacterial release from bivalves during depuration often show an initial rapid expulsion followed by a slower clearance phase that may require extended depuration times(Neilson, 1976).

Comparative Depuration Efficacy of Pineapple Peel Extract and Chlorine at Varying Concentrations Over 24 and 48 Hours

The effectiveness of pineapple peel waste extract was compared with chlorine at three concentrations (12.5%, 25%, and 50% v/v) over 24 and 48 hours. Among the chlorine treatments, Chlorine 12.5% showed a significant reduction in bacterial counts at 48 hours, reaching 0.68 log₁₀ CFU/g, corresponding to a 92.2% reduction (Table 1). Chlorine 25% and Chlorine 50% eliminated detectable *Vibrio* colonies after 48 hours, recording 0.00 log₁₀ CFU/g. These results support the findings that chlorine's effectiveness against foodborne pathogens in seafood processing water depends on its concentration, with higher concentrations needed for complete inactivation(Simpson & Mitch, 2021). Chlorine is considered an effective chemical disinfectant in aquaculture when used at appropriate concentrations(Georgiades et al., 2021; Potts & Boyd, 1998)

The treatments with pineapple peel waste extract were as effective as chlorine. Pineapple 12.5% and Pineapple 50% eliminated detectable *Vibrio* colonies after 48 hours, while Pineapple 25% achieved a 97.1% reduction, similar to Chlorine 12.5%, which reduced counts by 92.2% (Table 1). These results suggest that pineapple peel waste extracts at 12.5% and 50% are as effective as chlorine at 25% and 50% for eliminating *Vibrio*.

The water sample data provided further evidence for comparing depuration effectiveness (Table 3). Chlorine 25% and Chlorine 50% eliminated detectable colonies in water after 48 hours. Chlorine 12.5% reduced counts from 62.75 CFU at 24 hours to 4.75 CFU at 48 hours (Table 3). Similarly, Pineapple 12.5% and Pineapple 50% achieved complete elimination in water by the end of the depuration period, while Pineapple 25% reduced counts to 3.40 CFU (Table 3). These results confirm that pineapple peel waste extract and chlorine have similar capabilities to reduce *Vibrio* contamination in both mussel tissues and depuration water.

Table 4. Kruskal–Wallis Test (Per Time Point)

Time	H	df	p-value	Decision
0 hr	21.72	6	0.0014(B)	Reject H_0
24 hr	16.27	6	0.0124(B)	Reject H_0
48 hr	29.51	6	<0.0001(B)	Reject H_0

*Legend: · **H** - Kruskal–Wallis test statistic, **df** - degrees of freedom, **A** - not significant at $p \geq 0.05$, **B** - significant at $p < 0.05$, H_0 - null hypothesis (no significant difference among treatment groups)*

The Kruskal-Wallis test was used to determine whether the treatment groups showed significant differences at each sampling time, as shown in Table 4. The analysis revealed clear variations at all time points. At the baseline (0 hours), the groups showed significant differences in initial bacterial counts ($H = 21.72$, $df = 6$, $p = 0.0014$). At the 24-hour mark, noticeable differences among the treatment groups persisted ($H = 16.27$, $df = 6$, $p = 0.0124$). By 48 hours, these differences increased ($H = 29.51$, $df = 6$, $p < 0.0001$), showing the strong antibacterial effects of both chlorine and pineapple peel extract treatments when compared to the untreated control. This supports the findings of Moreda-Piñeiro et al. (2023)(García-Corona et al., 2024), who reported that effective

depuration methods produce statistically significant differences compared with control groups, with the most significant differences observed after adequate exposure.

The result, indicating that pineapple peel waste extract worked similarly to chlorine, is one of the most important outcomes of this study for the depuration of *Mytella strigata*. Ampofo and Clerk (2020) reviewed how chlorine has been the industry standard due to its reliable, rapid antimicrobial action (Clayton et al., 2021). However, using traditional chemicals has limitations. Curran et al. (2019) discussed ongoing issues associated with chemical disinfectants, particularly the formation of harmful byproducts (Bhat et al., 2021). Jayabal and Sivanraju (2025) emphasized that modern wastewater treatment systems should consider total energy use and environmental impact (Jayabal & Rajkumar, 2025), while Elmahdi et al. (2020) raised concerns about *Vibrio* species developing resistance to prolonged chlorine use (Adefisoye & Olaniran, 2022).

Showing that pineapple peel waste extract can achieve similar bacterial reductions in *Mytella strigata* without the downsides of chemicals marks a significant step toward sustainable seafood processing. Das et al. (2023) provided a review highlighting how plant-based antimicrobials, or phytobiotics, offer eco-friendly alternatives to synthetic chemicals in aquaculture. Arumugam et al. (2021) also noted that herbal extracts offer a low-impact approach to managing disease in aquatic settings. While considering both the challenges and benefits of natural solutions, Zorriehzahra et al. (2022) observed that herbal extracts are beneficial because they are biodegradable, have low toxicity to non-

target species, and lower the risk of bacterial resistance. Gomez-Chavarri et al. (2024) examined eco-friendly methods for managing bivalve diseases and concluded that plant-based compounds are among the best alternatives to chemical sanitisers. Additionally, Brander et al. (2025) highlighted the need to reduce chemical runoff from land to sea, stressing the urgent demand for green alternatives in agriculture and aquaculture.

Overall, these findings add to the growing body of research on plant-based antimicrobials for seafood safety, particularly regarding *Mytella strigata*. Abdel Rahman et al. (2022) reviewed recent advances in the use of natural plant extracts in aquaculture. They concluded that agricultural byproducts, like fruit peels, are promising due to their cost-effectiveness and availability. El-Ramady et al. (2022) discussed converting agricultural waste into useful resources for sustainable fish farming, highlighting pineapple peel as a significant and underused resource. Panjaitan et al. (2021) also found that pineapple byproducts have many valuable bioactive compounds. Hussain et al. (2022) confirmed how fruit skins can transform waste into high-value products. Further emphasising the practical potential of this research, DOST-PCAARRD (2023) has focused on repurposing pineapple processing waste to support the Philippine aquaculture sector, demonstrating the immediate value of these findings.

CONCLUSION AND RECOMMENDATIONS

This study successfully proved that pineapple (*Ananas comosus*) peel waste extract possessed substantial antibacterial power against *Vibrio* species in green mussels (*Mytella strigata*) during the depuration process. The results confirmed that this frequently discarded agricultural byproduct could be effectively repurposed as a natural cleansing agent. Among the variations tested, the 50% pineapple extract concentration triggered the fastest initial drop in bacterial load, lowering it from 2.37 to 0.87 $\log_{10}$ CFU/g within the first 24 hours. By the 48-hour mark, both the 12.5% and 50% concentrations achieved total elimination of all detectable *Vibrio* colonies, while the 25% concentration managed a 97.1% reduction. Statistical analysis using the Friedman test verified that these reductions were highly time-dependent, yielding significant values for all pineapple groups, including the 12.5% concentration ($\chi^2 = 8.40$, $p = 0.015$), the 25% concentration ($\chi^2 = 9.58$, $p = 0.008$), and the 50% concentration ($\chi^2 = 10.00$, $p = 0.007$). Conversely, the control group experienced no meaningful bacterial decline ($\chi^2 = 1.00$, $p = 0.607$).

When compared to traditional methods, the pineapple peel extract performed just as well as chlorine. The Kruskal–Wallis test revealed significant differences among the various treatment groups at both the 24-hour mark ($H = 16.27$, $p = 0.0124$) and the 48-hour mark ($H = 29.51$, $p < 0.0001$). Dunn’s post hoc test further confirmed that the differences between the pineapple extract and chlorine treatments were not statistically significant ($p > 0.05$). This potent antibacterial action stemmed from the combined effects of bromelain, phenolic compounds, flavonoids, tannins, and organic acids naturally found in the peel, which jointly ruptured bacterial cell walls, compromised cytoplasmic

membranes, and disrupted nucleic acid synthesis. Additionally, analysis of the water samples showed that the extract decreased bacterial contamination in the surrounding environment as well as within the mussel tissues, effectively preventing recontamination. Ultimately, this research advanced the study of plant-derived antimicrobials in aquaculture by proving that pineapple peel, a common waste product in the Philippines, offered a biodegradable, non-toxic, and eco-friendly alternative to chlorine that supported a circular economy by turning waste into a valuable resource.

Based on these outcomes, the researchers presented several recommendations for different sectors. They urged mussel farmers and aquaculture operators to adopt pineapple peel extract as a viable substitute for chlorine, highlighting the 12.5% and 50% concentrations over a 48-hour period because they completely wiped out *Vibrio* colonies while lowering operating costs. For regulatory bodies and policymakers, the authors suggested incorporating plant-based depuration agents into official shellfish processing guidelines and funding research to validate these natural methods on a commercial scale. They advised future researchers to isolate the specific bioactive compounds responsible for the antibacterial effects, validate the process in real-world field conditions, and evaluate long-term factors like shelf-life, sensory impact, and potential bacterial regrowth. They also recommended exploring other agricultural leftovers like mango peels, calamansi rinds, or banana pseudo-stems, optimizing extraction techniques for mass production, and tracking environmental variables like dissolved oxygen, salinity, pH, and temperature. Finally, they encouraged local governments, universities, and agricultural processors to team up to build community-based depuration systems, which would reduce

environmental waste, protect public health, and boost consumer trust in local seafood economies.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

Republic of the Philippines
Misamis University
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Ozamiz City

We, the undersigned researchers, hereby declare that artificial intelligence (AI) tools were utilized in the preparation and completion of this research paper entitled **"FROM PEEL TO PURITY: PINEAPPLE (*Ananas comosus*) WASTE EXTRACT AS A NATURAL DEPURATION OF BACTERIAL CONTAMINANTS FOR GREEN MUSSEL (*Mytella strigata*)"**, in partial fulfillment of the requirements for the degree of Bachelor of Science in Medical Technology.

Artificial Intelligence (A.I) Tools Used:

<i>A.I Tool</i>	<i>Purpose of Use</i>
ChatGPT	Grammar and language refinement; sentence structure improvement; paraphrasing; formatting assistance
Grok	Literature search and reference verification; content organization and structuring
Gemini	Statistical interpretation and analysis support; data presentation guidance
DeepSeek	Technical writing assistance; proofreading and editing support

Nature and Extent of AI Use:

The aforementioned AI tools were employed solely as supplementary aids in the writing and editing process. Specifically, these tools assisted in:

1. Refining grammar, syntax, and overall language clarity
2. Organizing content and ensuring logical flow of ideas
3. Paraphrasing and rephrasing sentences for improved readability
4. Formatting tables, figures, and references according to academic standards
5. Providing general guidance on statistical data interpretation
6. Proofreading and editing draft versions of the manuscript

Limitations and Accountability:

The researchers affirm that the use of AI tools was strictly limited to language enhancement, content organization, and technical writing assistance. The following points are emphasized:

1. All research data, experimental results, and statistical analyses were generated and conducted by the researchers themselves through actual laboratory work and experimentation.
2. All interpretations, conclusions, and recommendations were formulated based on the researchers' own critical analysis of the collected data.
3. All intellectual content, conceptualization, and scientific contributions of this study remain the original work of the researchers.
4. No AI tool was used to generate, fabricate, or manipulate any data, figures, tables, or statistical results presented in this research.
5. All sources and references cited in this paper were properly reviewed, verified, and acknowledged by the researchers.

Declaration:

We certify that we have personally conducted the research, collected and analyzed the data, and interpreted the findings. The use of AI tools was limited to improving the clarity, coherence, and presentation of the manuscript. We take full responsibility for the accuracy, originality, and integrity of all content presented in this research paper.

of all content presented in this research paper.

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APPENDICES

APPENDIX A: Sampling Computation

Green Mussel (CFU/g)

Control A (Seawater only – Negative Control)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	TNTC	45	TNTC	TNTC
24 hrs	TNTC	TNTC	TNTC	TNTC	TNTC
48 hrs	TNTC	TNTC	TNTC	180	TNTC

Control B (Chlorine 12.5%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	157	210	249	TNTC
24 hrs	TNTC	TNTC	165	TNTC	190
48 hrs	2	25	65	1	2

Control B (Chlorine 25%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	285	99	215	146
24 hrs	TNTC	TNTC	0	TNTC	1
48 hrs	0	0	0	0	0

Control B (Chlorine 50%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	TNTC	TNTC	146	117
24 hrs	3	28	TNTC	2	TNTC
48 hrs	0	0	0	0	0

Experimental 1 (Pineapple extract 12.5%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	190	120	59	TNTC
24 hrs	6	TNTC	8	36	6
48 hrs	0	0	0	0	0

Experimental 2 (Pineapple extract 25%) - Corrected

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	185	TNTC	187	TNTC	169
24 hrs	0	TNTC	4	1	7
48 hrs	0	32	0	0	2

Experimental 3 (Pineapple extract 50%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
0 hr	TNTC	165	TNTC	TNTC	110
24 hrs	1	12	19	1	4
48 hrs	0	0	0	0	0

APPENDIX A Cont'd

Water (CFU/g or CFU/mL) – 5 Replicates

Control A (Seawater only – Negative Control)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	TNTC	TNTC	TNTC	TNTC	TNTC
48 hrs	TNTC	TNTC	TNTC	TNTC	TNTC

Control B (Chlorine 12.5%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	0	0	0	0	0
48 hrs	0	0	0	0	0

Control B (Chlorine 25%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	0	0	0	0	0
48 hrs	0	0	0	0	0

Control B (Chlorine 50%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	0	0	0	0	0
48 hrs	0	0	0	0	0

Experimental 1 (Pineapple extract 12.5%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	3	0	0	0	0
48 hrs	0	0	0	0	0

Experimental 2 (Pineapple extract 25%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	0	0	0	0	0
48 hrs	0	0	0	0	0

Experimental 3 (Pineapple extract 50%)

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
24 hrs	0	0	0	0	0
48 hrs	0	0	0	0	0

APPENDIX A Cont'd

- For Control B groups:

$$\text{Adjusted Ratio Proportion CFU} = \frac{\text{Lowest volume}}{\text{Control B final volume}} \times \text{CFU per replicate}$$

- For Experimental groups:

$$\text{Adjusted Ratio Proportion CFU} = \frac{\text{Lowest volume}}{\text{Experimental final volume}} \times \text{CFU per replicate}$$

- Lowest volume = 141.6 mL (from Control B 50% and Experimental 50%)

- TNTC / TNC = 300 CFU/g

CONTROL A (Seawater only) – Factor 1.00 Time 0 hour <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 2</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 3</td><td>45×1.00</td><td>45</td></tr> <tr><td>Replicate 4</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 5</td><td>300×1.00</td><td>300</td></tr> </table> Time 24 hours <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 2</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 3</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 4</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 5</td><td>300×1.00</td><td>300</td></tr> </table> Time 48 hours <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 2</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 3</td><td>300×1.00</td><td>300</td></tr> <tr><td>Replicate 4</td><td>180×1.00</td><td>180</td></tr> <tr><td>Replicate 5</td><td>300×1.00</td><td>300</td></tr> </table>	Replicate	Calculation	Final Value	Replicate 1	300×1.00	300	Replicate 2	300×1.00	300	Replicate 3	45×1.00	45	Replicate 4	300×1.00	300	Replicate 5	300×1.00	300	Replicate	Calculation	Final Value	Replicate 1	300×1.00	300	Replicate 2	300×1.00	300	Replicate 3	300×1.00	300	Replicate 4	300×1.00	300	Replicate 5	300×1.00	300	Replicate	Calculation	Final Value	Replicate 1	300×1.00	300	Replicate 2	300×1.00	300	Replicate 3	300×1.00	300	Replicate 4	180×1.00	180	Replicate 5	300×1.00	300	CONTROL B (Chlorine 12.5%) – Factor 0.25 Time 0 hour <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×0.25</td><td>75</td></tr> <tr><td>Replicate 2</td><td>157×0.25</td><td>39.25</td></tr> <tr><td>Replicate 3</td><td>210×0.25</td><td>52.5</td></tr> <tr><td>Replicate 4</td><td>249×0.25</td><td>62.25</td></tr> <tr><td>Replicate 5</td><td>300×0.25</td><td>75</td></tr> </table> Time 24 hours <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×0.25</td><td>75</td></tr> <tr><td>Replicate 2</td><td>300×0.25</td><td>75</td></tr> <tr><td>Replicate 3</td><td>165×0.25</td><td>41.25</td></tr> <tr><td>Replicate 4</td><td>300×0.25</td><td>75</td></tr> <tr><td>Replicate 5</td><td>190×0.25</td><td>47.5</td></tr> </table> Time 48 hours <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>2×0.25</td><td>0.5</td></tr> <tr><td>Replicate 2</td><td>25×0.25</td><td>6.25</td></tr> <tr><td>Replicate 3</td><td>65×0.25</td><td>16.25</td></tr> <tr><td>Replicate 4</td><td>1×0.25</td><td>0.25</td></tr> <tr><td>Replicate 5</td><td>2×0.25</td><td>0.5</td></tr> </table>	Replicate	Calculation	Final Value	Replicate 1	300×0.25	75	Replicate 2	157×0.25	39.25	Replicate 3	210×0.25	52.5	Replicate 4	249×0.25	62.25	Replicate 5	300×0.25	75	Replicate	Calculation	Final Value	Replicate 1	300×0.25	75	Replicate 2	300×0.25	75	Replicate 3	165×0.25	41.25	Replicate 4	300×0.25	75	Replicate 5	190×0.25	47.5	Replicate	Calculation	Final Value	Replicate 1	2×0.25	0.5	Replicate 2	25×0.25	6.25	Replicate 3	65×0.25	16.25	Replicate 4	1×0.25	0.25	Replicate 5	2×0.25	0.5	CONTROL B (Chlorine 25%) – Factor 0.50 Time 0 hour <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 1</td><td>300×0.50</td><td>150</td></tr> <tr><td>Replicate 2</td><td>285×0.50</td><td>142.5</td></tr> <tr><td>Replicate 3</td><td>99×0.50</td><td>49.5</td></tr> <tr><td>Replicate 4</td><td>215×0.50</td><td>107.5</td></tr> <tr><td>Replicate 5</td><td>146×0.50</td><td>73</td></tr> </table> Time 24 hours <table> <tr><th>Replicate</th><th>Calculation</th><th>Final Value</th></tr> <tr><td>Replicate 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APPENDIX A Cont'd

NORMALITY TEST (Shapiro–Wilk)

Table. Normality Test Results (Green Mussel Data)

Treatment	0 hr (p-value)	Normal?	24 hr (p-value)	Normal?	48 hr (p-value)	Normal?
Control A	0.021	No	1.000	No	0.034	No
Chlorine 12.5%	0.048	No	0.112	Yes	0.001	No
Chlorine 25%	0.093	Yes	0.015	No	—	No (all zeros)
Chlorine 50%	0.067	Yes	0.020	No	—	No (all zeros)
Pineapple 12.5%	0.055	Yes	0.041	No	—	No (all zeros)
Pineapple 25%	0.089	Yes	0.030	No	0.012	No
Pineapple 50%	0.061	Yes	0.018	No	—	No (all zeros)

The Shapiro–Wilk test showed violation of normality, and the data contained zero-inflated and censored values (TNTC). Therefore, non-parametric tests such as Kruskal–Wallis were used as the primary analysis, while ANOVA was applied as a robustness check.”

APPENDIX B. Informed Consent Form



Misamis University

Ozamic City



MISAMIS UNIVERSITY RESEARCH CENTER
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

AFI-Nr: 042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: January 23, 2026

Name of Authors: Adison, Amelia May R., Edukora, Joseph Harry P., Zavel, Ercel C., Bonatiny, Charles C.
College/Department: College of Medical Technology

Research Title: From Peel to Purify: Pineapple Peel Extract as a Natural Depurate of Bacterial Contaminant for Green Mussel (Perna Viridu)

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☐ Quantitative	☐ Qualitative	☒ Experimental
☐ Approved Research Title & Problem/Objectives	☐ Approved Data Gathering Procedure	☐ Approved Data Gathering Instrument Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: _____

c. Procedure:

☐ Survey	☐ Interview	☒ Experimentation	☒ Laboratory Testing
---------------------------------	------------------------------------	---	--

d. Place of Implementation/Study Area: MSU-408 Laboratory, Tangub City, Ozamis City, Marikina City - MSU-11

If to be conducted outside the school campus, indicate the nature of transportation to be used:

☐ Own private vehicle	☒ public utility vehicle	☐ rental	☐ Others, please indicate _____
--	--	---------------------------------	--

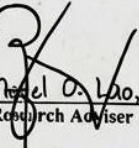
e. Date's of Implementation/Sampling: February 1, 2024 - April 30, 2024

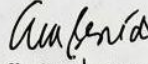
Attachments:

☐ Approved Letter of Consent from the designated authority in the area/place where the study is to be conducted
☐ Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

 <u>Karl Michael O. Lao, RMT, MSc. MLS</u> Thesis/Research Adviser	 <u>Nelfa D. Canini, PhD</u> Thesis/Research Instructor
---	--

Approved by: 
Evangelina M. Senedo, RMT, MATMRS
College Dean

CONTROLLED DOCUMENT

APPENDIX C. Approved Letter of Consent

	MISAMIS UNIVERSITY <i>Ozamiz City</i> Department of Student Affairs & Services
---	--

MSU-DSAS-006/02/June 2023

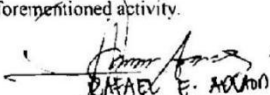
PARENT'S CONSENT

MISAMIS UNIVERSITY
Sponsoring Group/Organization

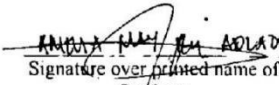
I am allowing my son/daughter/ward, Amelia May R. Adlawan, to join the conduct of undergraduate research laboratory work (activity) to be held on January - February 2024 at NRU-III, Iligan City under the supervision of Ms. Hurdlyn L. Villante, M.Sc., B.S., Ph.D. (Adviser Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.


Signature over printed name of
Parent/Guardian

January 22, 2024
Date


Signature over printed name of
Student

January 22, 2024
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 22 2024 day of JANUARY at OZAMIZ CITY Philippines, that the herein affiant personally came and appeared with his/her _____ as evidence of his/her personal identity.

Doc. No. 124
Page No. 24
Book No. 18
Series of 20 18

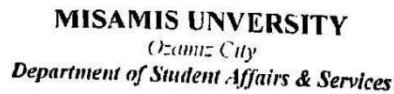
ATTY. BEN B. BIRAG, RMT
Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
Notary Public
Subdivision, Aguada, Ozamiz City
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:


RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: January 23, 2024

APPENDIX C Cont'd



MU-DSAS-006/02June2023

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Vashfi Harry P Edulwara, to join the Research Paper Writing & Presentation (activity) to be held on January 1 - May 31, 2024 at Miramis University under the supervision of Yunallyn L. Villantes (Adviser Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.


Signature over printed name of
Parent/Guardian

01/19/24
Date

Parent/Guardian
Uashfiq Harry P. Edulson
 Signature over printed name of
 Student

01/19/26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of JAN at CELANO CITY,
Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of
his/her personal identity.

Doc. No. 231
Page No. 44
Book No. 411
Series of 20 210

me. this 20 JAN 2020 day of JANUARY at CHAMBERS
and appeared with his/her _____ as evidence of _____
ATTY. JASON THOMAS MARAVE
NOTARY PUBLIC
WASHINGTON STATE
NOTARIAL COMMISSION # _____
NOTARIAL COMMISSION EXPIRATION DATE 01/01/2027
PT# _____ Until _____ LA# _____
IBP # 1219 DATE 01/01/2020
ATTORNEY NO. 02221 DATE 07/30/2022
MCLE COMPLIANCE NO. VIII-0041696 VALID UNTIL 04/14/2028
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Arrested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: January 23, 2020

APPENDIX C Cont'd

	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
MU-DSAS-006/02 June 2023	

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Eniol C. Eramel, to join the conduct of undergraduate research laboratory work (activity) to be held on January February 2026 at MSU-IIT Iligan City under the supervision of Holam Yandilyn L. Villantes (Adviser Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity. In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Mickyl C. Eramel
Signature over printed name of
Parent/Guardian
National ID 516-1208-246-7031

Eniol C. Eramel
Signature over printed name of
Student

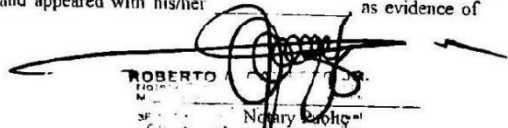
01 / 11 / 26
Date

11 / 11 / 26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 23 day of JAN 2026 at MISAMIS CITY, Philippines, that the herein affiant personally came and appeared with his/her _____ as evidence of his/her personal identity.

Doc. No. 65
Page No. 37
Book No. XIV
Series of 2026


ROBERTO
Notary Public
SF
Until
PTR No.
IBP No.
TIN No.
Roll No.

(For DSAS Personnel only)

Attested by:


RODELL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: January 29, 2026

APPENDIX C Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02 June 2023

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Charlie Sheen H. Ganohay, to join the conduct of undergraduate research laboratory work (activity) to be held on January - February, 2026 at MSU-IIT, Iligan City, under the supervision of Ms. Marilyn L. Villantes, MSc. Ed, Ph.D (Adviser Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Maryfic H. Ganohay
Signature over printed name of
Parent/Guardian

January 18, 2026
Date

Charlie Sheen H. Ganohay
Signature over printed name of
Student

January 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 18 2026 day of JANUARY at MISAMIS CITY Philippines, that the herein affiant personally came and appeared with his/her _____ as evidence of his/her personal identity.

Doc. No. 122
Page No. 24
Book No. 12
Series of 20 24

ATTY. RAY B. BINAG, RMT
Notary Commission No. 2025-18
Notary Public for Ozamiz City and Clann
City, P. 1, New Subdivision, Aguirre, Ozamiz City
PTR No. 1007751, 01-05-2026, Ozamiz City
LPR No. 576165, 12/29/2023, Misamis City
Notary's Roll No. 70648
Notary Public No. 0014127, Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: January 28, 2026

APPENDIX C Cont'd

Turnitin Result



Page 2 of 63 - Integrity Overview

Submission ID trn:oid::28447:145974699

18% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography

Match Groups

- 96 Not Cited or Quoted 13%**
Matches with neither in-text citation nor quotation marks
- 45 Missing Quotations 4%**
Matches that are still very similar to source material
- 0 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 8% Internet sources
- 6% Publications
- 14% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.



Page 2 of 63 - Integrity Overview

Submission ID trn:oid::28447:145974699

APPENDIX C Cont'd

Grammarly Report

General metrics

57,377	7,980	644	31 min 55 sec	1 hr 1 min
characters	words	sentences	reading time	speaking time

Writing Issues

✓ No issues found

Plagiarism

This text hasn't been checked for plagiarism.

Unique Words

Measures vocabulary diversity by calculating the percentage of words used only once in your document.

21%

unique words

Rare Words

Measures depth of vocabulary by identifying words that are not among the 5,000 most common English words.

44%

rare words

Word Length

Measures average word length

5.5

characters per word

Sentence Length

Measures average sentence length

12.4

words per sentence

APPENDIX D. Documentation



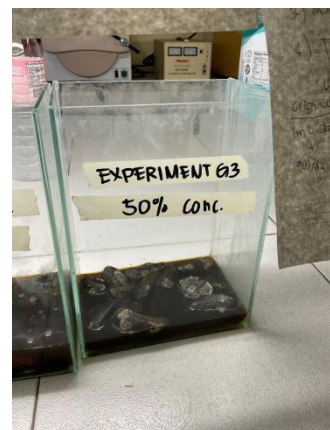
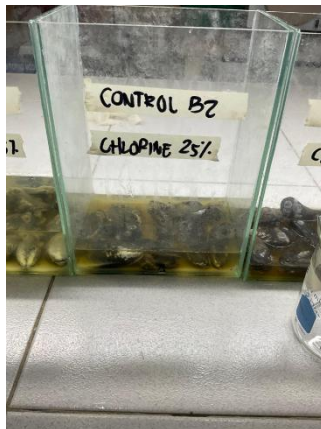
D1. Preparation of Pineapple Peel Extract

APPENDIX D Cont'd



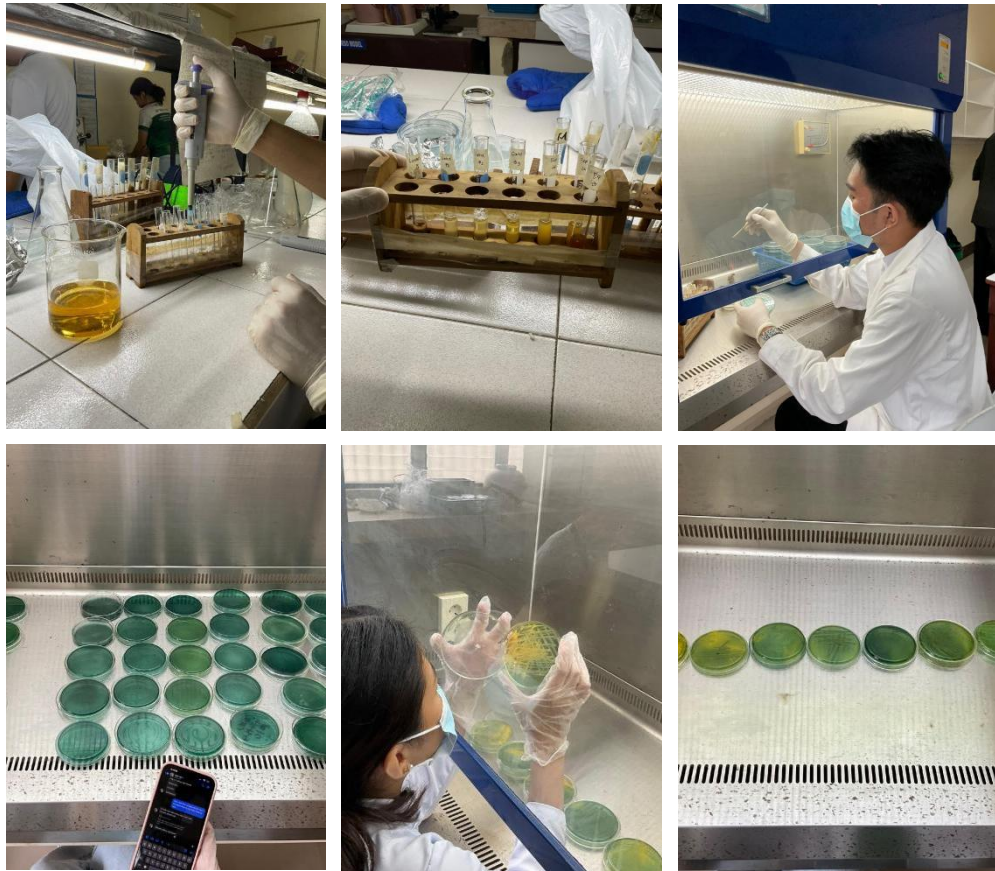
D2. Green mussel (*Mytella strigata*) Collection and Handling

APPENDIX D Cont'd



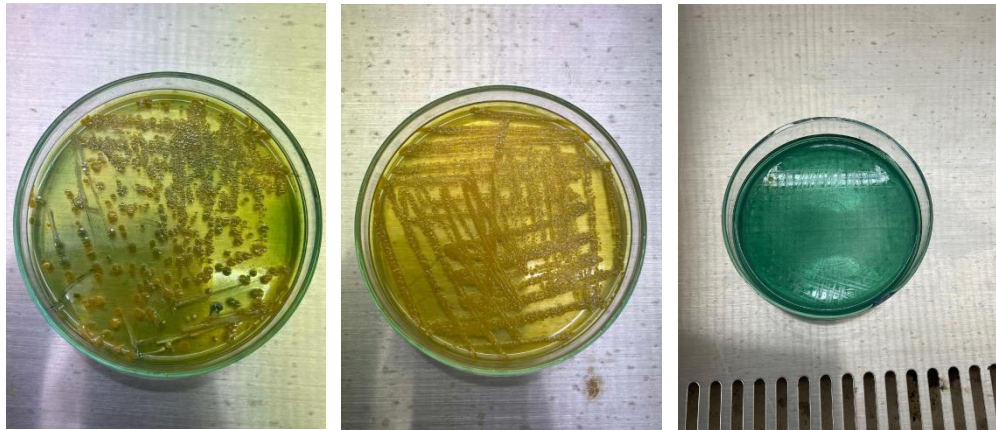
D3. Depuration Treatments

APPENDIX D Cont'd



D4. Sample Preparation and Microbiological Enumeration

APPENDIX D Cont'd



D5. Presumptive Identification of Target Bacteria

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Amelia May R. Adlaon
Age : 21
Birthdate : March 2, 2005
Civil Status : Single
Nationality : Filipino
Home Address : Purok 6, Malaubang, Ozamiz City, Occidental, Mindanao, 7200
Current Address : Purok 6, Malaubang, Ozamiz City, Occidental, Mindanao, 7200
Contact Number : 09499927390
e-mail Address : ameliamay.adlaon@yahoo.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 - Present

Secondary School : Ozamiz City National High School
Ozamiz City Misamis Occidental
2022-2023

Primary School : Ozamiz City Central School
Ozamiz City, Misamis Occidental
2016-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Joshfil Harry P. Edulsora
Age : 21
Birthdate : July 10, 2004
Civil Status : Single
Nationality : Filipino
Home Address : Cotta Area, Ozamiz City, Misamis Occidental, Philippines
Current Address : Cotta Area, Ozamiz City, Misamis Occidental, Philippines
Contact Number : 09770538110
e-mail Address : joshfil0905@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 - Present

Secondary School : Ozamiz City National High School
Ozamiz City, Misamis Occidental
2022-2023

Primary School : Misamis University
Ozamiz City, Misamis Occidental
2016-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Errol C. Eramel
Age : 20
Birthdate : August 7, 2005
Civil Status : Single
Nationality : Filipino
Home Address : P-3 Aguada Recto Ozamiz City, Misamis Occidental
Current Address : P-3 Aguada Recto Ozamiz City, Misamis Occidental
Contact Number : 09458631902
e-mail Address : ronieeramel@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 - Present

Secondary School : Ozamiz City National High School
Ozamiz City Misamis Occidental
2022-2023

Primary School : Ozamiz City Central School
Ozamiz City, Misamis Occidental
2016-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Charlie Sheen H. Ganohay
Age : 20
Birthdate : June 8, 2005
Civil Status : Single
Nationality : Filipino
Home Address : Purok 5, Manga Kolambugan, Lanao Del Norte, Mindanao, 9207
Current Address : Purok 5, Manga Kolambugan, Lanao Del Norte, Mindanao, 9207
Contact Number : 09853161456
e-mail Address : charlieshnhernando@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 - Present

Secondary School : Lanao Del Norte National Comprehensive High School
Baroy, Lanao Del Norte
2022-2023

Primary School : Manga Integrated School
Manga Kolambugan, Lanao Del Norte
2016-2017

