DEVELOPMENT OF A DURIAN *(Durio zibethinus)* PULP-BASED GENERAL PURPOSE SOLID CULTURE MEDIUM FOR COMMON PATHOGENIC BACTERIA

A RESEARCH PAPER

Presented to
the Faculty of the College of Medical Technology
Misamis University
Ozamiz City

In Partial Fulfillment
of the Requirements for the Degree
BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY

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JUNE 2026

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ABSTRACT

While commercial agar media are essential in microbiology, their cost and limited availability may restrict accessibility in some settings. This study investigated durian *(Durio zibethinus*) pulp as a potential component of an alternative solid culture medium for bacterial growth. No prior studies have extensively evaluated durian pulp-based agar for cultivating common pathogenic bacteria, making this study a preliminary investigation. An experimental comparative design was used to formulate durian-based agar at 5%, 10%, and 15% concentrations combined with bacteriological agar powder as a solidifying agent. The test organisms included *Staphylococcus aureus (*ATCC 25923)*, Escherichia coli* (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Nutrient agar served as the positive control. Data were analyzed using descriptive statistics and comparative analysis based on semi-quantitative growth intensity scoring and colony morphology assessment. Results showed that all test organisms grew on the durian-based agar, but growth intensity was lower than on nutrient agar. Mean growth scores on durian-based media ranged from 1.0 to 2.0, while nutrient agar consistently showed strong growth (mean = 3.0). Among the formulations, the 15% concentration showed relatively better growth performance. Colony morphology on durian-based agar was generally effused, faint, and less distinct, while nutrient agar produced well-defined colonies.Overall, durian pulp-based agar supported basic bacterial growth but showed limitations in growth intensity and colony differentiation. These findings suggest potential for development as a sustainable culture medium, though further optimization is needed to improve performance.

Keywords: *alternative growth medium, bacteriological advancement, differential microbiology, economical formulation, fruit-derived agar*

DEDICATION

This research is respectfully and wholeheartedly dedicated to our dear **parents**, who have been the source of our strength and inspiration throughout our academic path with their endless love, sacrifices, and steadfast support. Your support, leadership and continuous prayers have inspired us to overcome hurdles and excel in all we do.

This work is also dedicated to our instructors, mentors, and friends who have graciously contributed their knowledge, wisdom, and encouragement. Without your direction and support, this project would not have been possible. You have inspired us to keep learning and growing.

Most importantly, we dedicate our research to Almighty God for His infinite gifts, wisdom, and guidance. His grace has carried us through this trip and made completion of this job possible.

ACKNOWLEDGMENT

First and foremost we thank and praise the Almighty God from the bottom of our hearts for his never-ending love, guidance, wisdom and blessings along this journey. In doubt, He gave us strength, in hardship, perseverance and hope. This research was made possible by his grace and divine guidance.

We are most grateful to our beloved parents for their unconditional love, sacrifices, understanding and unwavering support. Their endless encouragement, prayers and confidence in us has been our biggest motivation. And for that we are eternally grateful, for every achievement we make is a reflection of the countless sacrifices they have made for us.

Our sincere thanks to our professors and teachers for their dedication, knowledge, and guidance that have been invaluable to our academic development. Their patience, encouragement and their openness to share their expertise has encouraged us to learn, improve and reach for excellence in our studies.

Special thanks to our research adviser, **Mr. Karl Maxel O. Lao, RMT, MSc. MLS** for his immeasurable guidance, patience and unending support throughout the conduct of this study. His positive suggestions, encouragement and confidence in our abilities encouraged us to overcome difficulties and to successfully complete this research. We are truly thankful for the time, energy and wisdom he so generously shared with us.

Thank you to our friends and classmates for being a part of this journey. Your encouragement, understanding and companionship helped to make the challenges lighter

and the successes more meaningful. We shared memories, laughter and support that will forever be cherished.

Finally, we would like to thank all those who have, in one way or another, collaborated in the realization of this research. Through words of encouragement, acts of kindness, or valuable assistance, your support has made a meaningful impact on our journey.

This research is not just a product of our hard work, but a testament of the love, guidance and support of the people who stood by us every step of the way. We offer our deepest and most sincere thanks to all of you.

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INTRODUCTION

Background of the Study

Culture media are important components in microbiology, providing nutrients and environmental conditions which promote microbial growth and differentiation. Conventional media like nutritional agar depend on purified peptones, extracts, and commercial agar, which are frequently costly and sourced from other countries (Jung, 2024). The pursuit of alternative, locally generated, and economical media has garnered interest, particularly in poor nations with constrained laboratory resources.

Recent research have shown the potential of fruit and plant components as nutritional alternatives in culture medium compositions. The study of (Gupta et al., 2020) effectively formulated banana peel extract agar that supports the growth of *Cryptococcus neoformans*, emphasizing that plant-derived substrates can sustain microbial metabolism owing to their abundant carbohydrate, protein, and mineral content. Also, a study shows that durian seeds can be used as a cheaper alternative to nutrient agar for growing Staphylococcus aureus, with the best growth at a 200 g concentration (Juariah, 2021). Mango, potato, tomato, and banana pulps have been evaluated as organic nutrition sources that promote bacterial proliferation (Shareef, 2019). The development of locally sourced and environmentally sustainable culture media aligns with the principles of green microbiology and circular bioeconomy. Utilizing agricultural by-products such as fruit peels and pulps not only reduces laboratory expenses but also minimizes organic waste, contributing to sustainable research practices (Akinsemolu, 2023). Moreover, the dependence on imported commercial media components can hinder continuous microbiological investigations in low-resource settings, emphasizing the need for cost-

effective, indigenous alternatives (Antranikian & Streit, 2022). The adoption of fruit-based substrates for culture media thus presents both ecological and economic advantages for developing nations.

Durian *(Durio zibethinus)* is a tropical fruit prevalent in Southeast Asia, especially in the Philippines. The pulp contains abundant carbohydrates, amino acids, vitamins, and trace minerals that may function as natural nutrition for bacterial proliferation (A Aziz & Mhd Jalil, 2019). However, durian pulp by itself does not possess the gelling properties necessary to establish a solid media. Hence, its partnership with commercial bacteriological agar powder can yield a stable, nutrient-dense solid medium. In medical microbiology, distinguishing common pathogenic bacteria, such as *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* is essential for diagnostic purposes (Carroll & Pfaller, 2024).

General-purpose culture media, such as Nutrient Agar, contain peptone, beef extract, and agar to support the growth of a wide variety of non-fastidious microorganisms without selective or differential agents (Prescott et al., 2020). Developing a durian pulp-based agar as an alternative to Nutrient Agar may offer a cost-effective and locally sourced medium suitable for general bacterial cultivation. Although numerous studies have investigated fruit-based media, including banana, mango, and tomato, there is a limited existing study focused on utilizing durian pulp as a nutrient source for the formulation of selective and differential culture media. Most prior studies concentrated solely on analyzing overall bacterial proliferation, neglecting to test its ability to differentiate bacterial groups based on metabolic characteristics. This highlights the necessity to investigate whether durian pulp, a locally prevalent and unexplored resource, might function as a sustainable substitute the

formulation of commercial nutrient agar. Selective and differential media are essential in clinical diagnostics, enabling microbiologists to identify and differentiate harmful organisms according to their metabolic characteristics. Mannitol salt agar and eosin methylene blue (EMB) agar exemplify media that utilize specific substrates and pH indicators to elucidate microbial metabolic variations (Willey et al., 2021). Recent advancements have aimed to emulate these selective principles through the use of plant-derived bases, integrating natural extracts with selective agents to attain similar performance (Gupta et al., 2025). This illustrates the viability of altering fruit-based formulations to execute differential diagnostic functions, hence facilitating the development of innovative, cost-effective media like durian pulp agar.

Therefore, this study will investigate the viability of durian pulp as a nutrient base in the development of a differential solid medium that facilitates the growth and differentiation of common pathogenic organism specifically *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Objectives of Study

This study aimed assess a solid culture medium from Durian *(Durio zibethinus)* pulp incorporated with bacteriological agar powder as a solidifying agent for the growth and differentiation of common pathogenic bacteria. Specifically, it aimed to:

1. Formulate a durian-based agar at 5%, 10%, and 15% (v/v) with bacteriological powder (CAS No. 9002-18-0) and evaluate their physical properties compared to commercially sold nutrient agar;
2. Assess the bacterial growth performance of Staphylococcus aureus (ATCC 25923) ,

Escherichia coli (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) on durian-based formulations and nutrient agar using qualitative growth intensity scoring; and

3. Compare the colony morphology and pigmentation of common pathogenic bacteria on durian-based agar and nutrient agar.

Significance of the Study

The purpose of this study was to contribute to the existing field of utilizing alternative and sustainable microbiological media that were essential in the bacterial growth and assessment. Developing a durian-based culture medium provides multiple benefits in different aspects. It utilized locally available durian pulp which reduces the dependency on high-cost media components that are usually imported. This study encouraged waste management by repurposing fruit pulp in research purposes. This provided a substitute experiment for teaching basic microbiology and media formulation. It also benefits innovation in diagnostics by demonstrating that fruit-based or indigenous plant materials can take the place of conventional media bases for bacterial differentiation. Furthermore, this study serves as a reference for future researchers for formulations using other alternative fruit substrates that are available locally for bacterial experiment.

Scope and Delimitations

This study was limited to the evaluation and development of durian pulp-based gar media for common pathogenic bacteria only. The bacterial species that were tested are limited to *Staphylococcus aureus (*ATCC 25923)*, Escherichia coli* (ATC 25922) and

Pseudomonas aeruginosa (ATCC 27853) obtained from the institutional laboratory of Misamis University. The study did not include the isolation of bacteria from clinical or even environmental samples. The investigation focused on the growth performance of bacteria on durian pulp-based agar in comparison with nutrient agar. It did not cover advanced biochemical profiling, antimicrobial susceptibility, or long-term storage properties of the medium. The purpose of the findings was to perform a preliminary assessment of the ability of durian pulp-based agar to support bacterial growth. This study was intended for laboratory scale validation and does not go beyond clinical diagnostic use.

MATERIALS AND METHODS

Research Design

This study used an experimental comparative design to formulate and assess a durian (*Durio zibethinus)* pulp-based solid culture medium as an alternative to commercial simple media for common pathogenic bacteria. The preparation combined durian pulp extract along with bacteriological agar powder to achieve a proper solidification during incubation, as the pulp alone lacks the gelling property required for culture media. The design differentiated three concentrations of durian extract (5%, 10% and 15%) against the commercial nutrient agar as a control for this experiment.

The capability of formulated durian pulp-based media to support the growth of common pathogenic bacteria was assessed. This approach evaluated the bacterial growth based on colony appearance and morphology. Descriptive observation was used to compare bacterial growth performance and diagnostic characteristics relative to the standard medium.

The experimental procedure for testing the effectiveness of *Durio zibethinus* pulp-based agar media on common pathogenic bacteria were systematically carried out in stages, including preparation of materials, media formulation, inoculation and observation. The overall process is summarized

in the flowchart below (Figure 1).

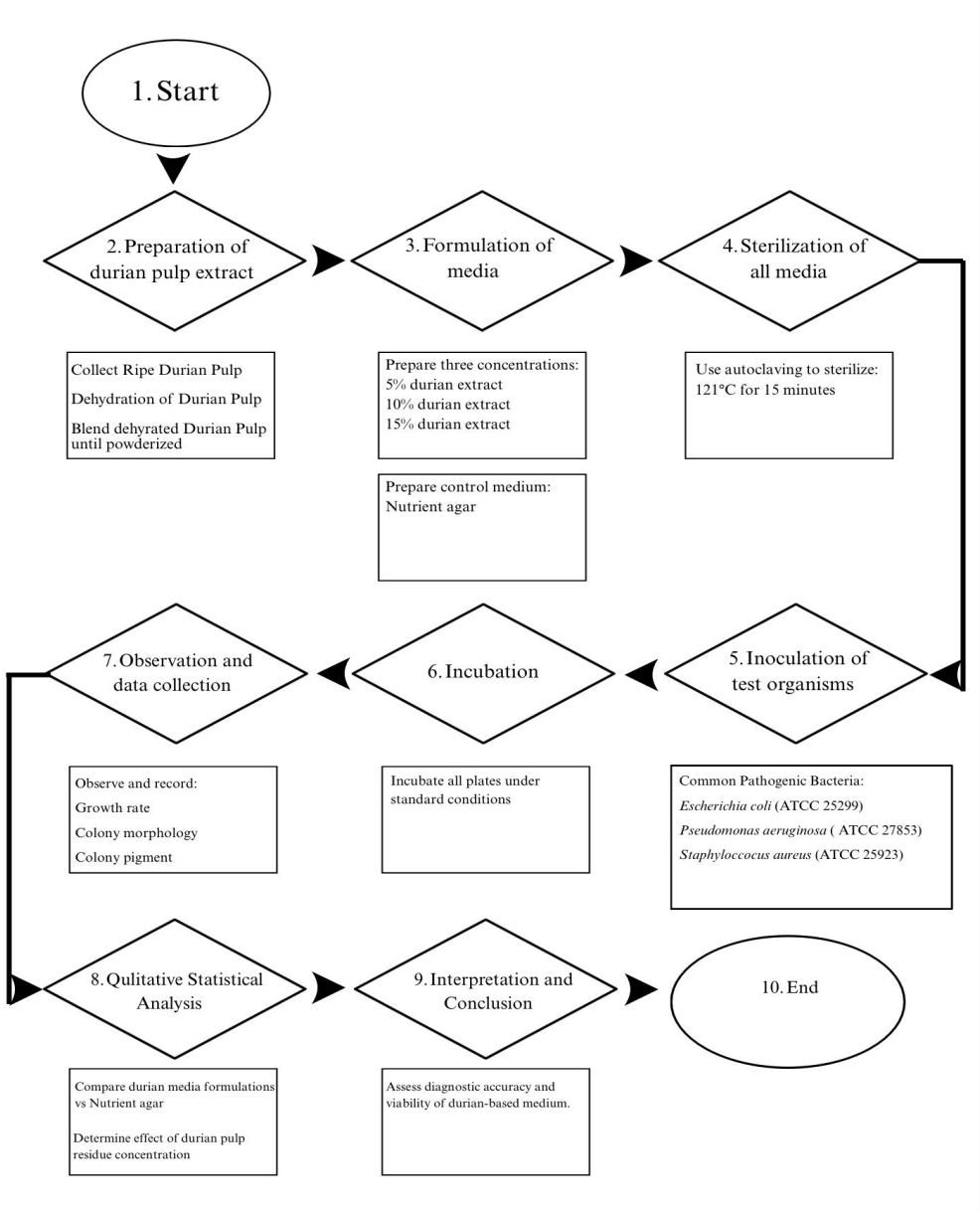

Figure 1. Flowchart of the experimental procedure for testing durian *(Durio zibethinus)* pulp-based agar medium on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Research Setting

The research was conducted at the Microbiology Laboratory of the Deparment of Medical Technology, Misamis University, ensuring a maintained Biosafety Level 2 classification that was suitable for handling pathogenic bacterial strain. The laboratory was equipped with vital facilities including an autoclave, incubator, biosafety cabinet, analytical balance, and sterile glassware required for the assessment of culture media preparation and cultivation of bacteria. The research spanned from January to April 2026. To minimize contamination, all procedures were performed under strict aseptic conditions within a certified biosafety cabinet, following institutional biosafety protocols and the World Health Organization (WHO) Laboratory Biosafety Manual guidelines. Contamination control measures included routine surface decontamination, sterilization of instruments and materials, proper use of personal protective equipment (PPE), adherence to good microbiological practices and procedures (GMPP), and proper waste segregation and disposal to prevent cross-contamination and exposure to biological hazards (Kojima et al., 2021).

Sample Collection

Reference strains of of *Staphylococcus aureus (*ATCC 25923) , *Escherichia coli* (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from the microbiology stock culture collection of the Department of Medical Technology Science, Misamis University. These pure bacterial reference strains were utilized as control organisms to evaluate the growth of the formulated durian pulp-based solid culture media. Each bacterial strain were subcultured onto freshly prepared nutrient agar (CAS No. 9002-18-0), incubated at 37°C for 24 hours before experimentation. Pure cultures of

each test organism were used. For each organism, a single colony exhibiting distinct morphological characteristics was selected and standardized to a 0.5 McFarland using sterile normal saline prior to inoculation.

Sample Preparation

Preparation of Durian Extract

Fresh durian fruits *(Durio zibethinus)* were sourced from local suppliers in Ozamiz City. After gathering the materials, the pulp were separated from the seeds using sterile forceps following established durian pulp preparation methods (Wasnin et al., 2012). The collected pulp was placed in a dehydrating oven at 60 °C for 48 hours until completely dried to inhibit microbial growth and facilitate powder production. The dried pulp was then ground into powder using a sterile blender to obtain finer and uniform particles (Herawati et al., 2025). To ensure no mold growth, the residue undergone drying inside a dehydrating oven for 48 hours under 60 degrees c. This step allowed the pulp to be powderized using a sterilized blender (Ankeli et *al*., 2025).

The availability of local durian ensured the use of fresh raw material while maintaining the integrity of the agar formulation.The same preparation steps were followed for both fresh and frozen pulp to maintain methodological consistency. To formulate the durian-pulp-based medium, varying concentrations of durian extract (5%, 10%, and 15% v/v) were combined with bacteriological agar powder (15 g/L). The mixture was heated until fully dissolved, sterilized by autoclaving at 121 °C for 15 minutes, and poured into sterile Petri plates under aseptic conditions. The prepared medium was then stored at 4 °C until use (Brown., 2010).

Preparation of Bacteriological Agar Base

To ensure a proper consistency in this study (CAS No. 9002-18-02) a solidifying agent was used. This consists of purified polysaccharides derived from red algae and serves as a neutral gelling substrate that does not interfere with bacterial metabolism (Cebrian et al., 2024). Three formulations were prepared:

Formula A: 5% v/v durian extract + 15 g/L agar

Formula B: 10% v/v durian extract + 15 g/L agar

Formula C: 15% v/v durian extract + 15 g/L agar

Different mixture of formulas was heated with constant stirring until the agar was completely dissolved. The prepared mixtures were sterilized by autoclaving at 121 °C for 15 minutes. After cooling to approximately 50 °C, the molten medium was poured onto sterile Petri dishes (20 mL/plate) and was allowed to solidify. A commercial nutrient was prepared following the manufacturer's instructions and was used as the control medium.

The preparation of the durian-based agar medium followed a systematic procedure to ensure consistency and suitability for the study. The process involved the preparation of the necessary materials, extraction and processing of the durian, formulation of the agar medium, sterilization, and final preparation before use. The overall procedure is summarized in the flowchart presented in Figure 2.

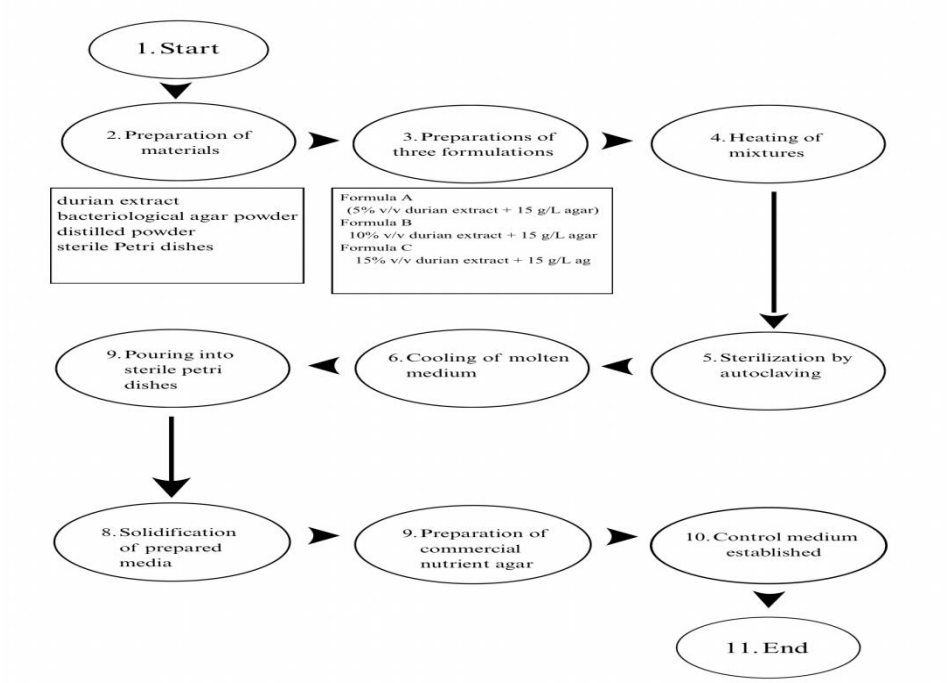

Figure 2. Flowchart of the preparation of experimental procedure for testing durian *(Durio zibethinus)* pulp-based *Bacterial Culture and Biochemical Testing*

Pure colonies of *Staphylococcus aureus (*ATCC 25923) , *Escherichia coli* (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were aseptically obtained from stock cultures and transferred into sterile normal saline solution to prepare bacterial suspensions. The suspensions were mixed thoroughly until a uniform turbidity comparable to the 0.5 McFarland standard was achieved (Tamma et al., 2022). Each bacterial suspension was then aseptically inoculated onto the surface of the prepared durian-based media and the control nutrient agar using the quadrant streak plate method. Inoculated plates were incubated at 37 °C for 24–48 hours. The study utilized 45 experimental setups (3 durian concentrations x 3 bacterial species x 5 replicates each). In addition, nutrient agar served as the control medium for each bacterial species with five

replicates, contributing 15 control for a total of 60 replicates to provide sufficient data for statistical analysis. Colony morphology, including size, shape, elevation, and margin, were examined and photographed for documentation. Results were compared between durian-based formulation and the control medium. To validate the results for common pathogenic bacteria, selected colonies ATCC 25922 (*Echerichia coli*), ATCC 27853 (*Pseudomonas aeruginosa*), and ATCC 25923 (*Staphyloccocus aureus*) were used as test organisms to ensure consistency and reliability of the results, while nutrient agar serves as the positive control. All plates were examined and photographed for documentation, and results were compared between durian-based formulations and the control medium.

Data Analysis

Data were analyzed using descriptive statistics (mean and standard deviation) based on semi-quantitative growth intensity scores from five replicates per treatment. Differences in growth intensity among concentrations were analyzed using the Kruskal–Wallis test at a 0.05 level of significance. The analyses was conducted to evaluate the physical properties, bacterial growth performance, and colony characteristics of durian-based agar enriched with bacteriological powder in comparison with commercial nutrient agar. The framework followed the approaches used by (Juariah, 2021) and (Afria et al., 2025), who both utilized durian seed derivatives as microbial growth media. The colony-forming unit (CFU) count was originally designed to provide a quantitative assessment of bacterial growth. However, the colony growth on the durian-based agar was confluent and effused, hence discrete colonies could not be formed. Therefore, qualitative growth

intensity grading was utilized instead of CFU counting.

The pH was measured using a calibrated pH meter, solidification time were recorded using a stopwatch from pouring to complete solidification, color were visually assessed under natural light, consistency was evaluated by gentle probing with a sterile applicator stick, and moisture content was determined by the loss-on-drying method using a dehydrating oven. It was subjected to descriptive statistical treatment by computing the mean and standard deviation for each concentration (5%, 10%, and 15%). The percentage of moisture was determined using the equation:

$$\%Moisture = \frac{p\,initial}{p\,dry} \times 100$$

Where:

W(initial) = weight of mixture before drying

W(dry) = weight of mixture after drying

This formula was used to determine the moisture content of the agar formulation to ensure that the durian-based agar had an appropriate consistency for bacterial growth. Excess moisture could lead to soft medium, while insufficient moisture could cause uneven solidification.

Biosafety and Biowaste

This study complied with university biosafety and ethical guidelines for the handling of bacterial cultures. The bacterial strains *Staphylococcus aureus (*ATCC 25923)*, Escherichia coli* (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were pathogenic reference organism obtained from standardized sources and handled under Biosafety Level 2 (BSL-2) laboratory conditions. The experiment took place in

the Microbiology Laboratory of the Department of Medical Technology, Misamis University that is furnished with a biosafety cabinet, autoclaves, and adequate waste management systems in accordance with WHO (2020) biosafety requirements. Appropriate personal protective equipment (PPE), including laboratory coats, gloves and surgical masks, was utilized throughout the experiment. All procedures involving bacterial cultures were conducted under aseptic conditions within the biosafety cabinet to reduce the risk of contamination and exposure. Contaminated culture plates, pipettes, and other materials were sterilized via autoclaving at 121 °C for 15 minutes prior to disposal, following standard biomedical waste management and biosafety procedures as recommended by the World Health Organization (Kojima et al., 2021).

RESULTS AND DISCUSSION

Physical properties of Durian-Based Agar

Agar from durian pulp successfully created a semi-solid medium at 5, 10 and 15% (v/v) concentrations. All formulations supported the growth of the test species, including *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* However, the growth of bacteria on the durian based medium was generally faint and less distinct than that of the nutrient agar control.

Table 1 shows the physical features of samples with different concentrations (5%, 10%, and 15%) in comparison with the nutrient agar control. A progressive change in color intensity was observed as concentration increased, from pale yellow to dark yellow. Opacity increased with concentration, from mildly opaque at lower concentrations to entirely opaque at 15%. This did not affect the consistency, which remained solid in all samples including the control. The usual reference condition was the nutrient agar control, which was clear amber and translucent. The results show that the increase of concentration mostly impacts the color and opacity, while the consistency remains constant.

Table 1. Physical Properties of Durian- Based Agar

Concentration	Color	Opacity	Consistency	Remarks
5%	Pale yellow	Slightly opaque	Firm	Stable
10%	Light yellow	Slightly opaque	Firm	Stable
15%	Dark yellow	Opaque	Firm	Stable
Nutrient Agar (Control)	Clear amber	Transparent	Firm	Standard

Figure 3 visually supports the findings presented in Table 1, showing progressive changes in color and opacity of durian-based afar formulations as concentrations increases. Similar results were found in previous studies using plant-based agar and polysaccharide gels. A study on durian fruit-hull polysaccharide gel revealed that the addition of durian-derived compounds had a significant effect on the structure of agar and exhibited antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* due to its bioactive sugar components (Lipipun et al., 2002). Moreover, extracts of durian seed showed inhibitory effects towards Gram positive and gram negativee bacteria, suggesting that durian contains compounds that can change the microbial environment and growth condition (Duazo, 2012).

Polysaccharide based formulations from durian were also reported to exhibit concentration dependent antimicrobial activity where higher concentrations led to stronger inhibitory effects and more significant physicochemical changes in the medium (Ponsamart et al., 2005). These findings support the present observation that the increasing concentration of durian not only affects the physical properties of the agar but may also enhance its potential bioactivity. In addition, similar concentration-dependent effects have been reported on other plant-based agar systems where higher levels of extracts lead to increased inhibition zones and changes in the characteristics of the medium due to higher levels of secondary metabolites such as flavonoids, tannins and phenolic compounds (Indrawati et al., 2024).

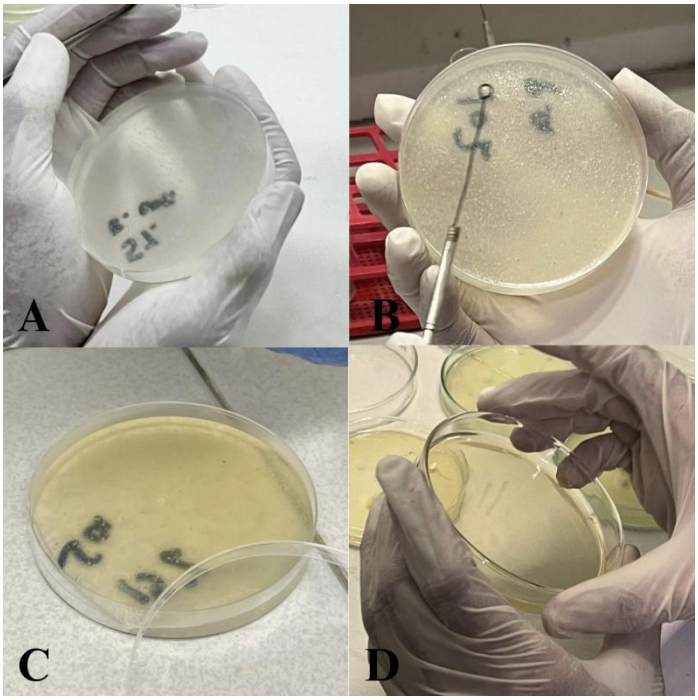

Figure 3. Physical appearance of durian-based agar formulations at varying concentrations (A-C: 5%, 10% and 15% respectively) compared with nutrient agar control (D)

Bacterial growth Performance on Durian-Based Agar and Nutrient Agar

The mean growth intensity scores of the test organisms on the durian-based agar formulations and the nutrient agar control are shown in Table 2. For comparison of bacterial growth performance at different concentrations, the scores were derived from a qualitative scale of growth intensity.

Table 2. Mean growth intensity scores (± standard deviation) of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa on durian-based agar formulations (5%, 10%, and 15%) and nutrient agar control using a semi-quantitative scoring system.

Bacteria	Medium	Concentration	Mean Growth Score ± SD	Interpretation
S. aureus	Durian-Based Agar	5%	1.0 ± 0.00	No to faint growth
S. aureus	Durian-Based Agar	10%	1.4 ± 0.32	Slight growth
S. aureus	Durian-Based Agar	15%	2.4 ± 0.55	Moderate growth
S. aureus	Nutrient Agar	*Control*	3.0 ± 0.00	Strong growth
E. coli	Durian-Based Agar	5%	1.0 ± 0.00	No to faint growth
E. coli	Durian-Based Agar	10%	1.2 ± 0.00	Slight growth
E. coli	Durian-Based Agar	15%	1.3 ± 0.00	Slight growth
E. coli	Nutrient Agar	*Control*	3.0 ± 0.00	Strong growth
P. aeruginosa	Durian-Based Agar	5%	1.0 ± 0.00	No to faint growth
P. aeruginosa	Durian-Based Agar	10%	1.1 ± 0.00	Slight growth
P. aeruginosa	Durian-Based Agar	15%	1.2 ± 0.00	Slight growth
P. aeruginosa	Nutrient Agar	*Control*	3.0 ± 0.00	Strong growth

Legend: Growth intensity was evaluated using a qualitative scoring system: 1.0 = no to faint growth, 1.1–1.6 = slight growth, 2.0 = moderate growth, and 3.0 = strong growth (nutrient agar control).

As shown, mean growth intensity scores indicated that all the test organisms were able to grow on the durian based agar formulations but the growth intensity was always lower than the nutrient agar control. At 5%, growth remained minimal (mean = 1.0 ± 0.00), while at 10% it slightly increased (mean = 1.4 ± 0.32), and further increased at 15% (mean = 2.4 ± 0.55) for Staphylococcus aureus. Also, *Escherichia col*i exhibited a progressive increase in growth intensity from 1.0 at 5% to 1.3 at 15%. *Pseudomonas aeruginosa* displayed a modest enhancement of growth at doses of 1.0 and 1.2. In contrast, the nutrient agar control continuously maintained robust bacterial growth (mean = 3.0) for all isolates. The presence of a standard deviation of 0.00 for most concentrations indicates highly consistent replicate results, suggesting uniform bacterial

response across trials. In contrast, higher standard deviation values observed in S. aureus at 10% and 15% indicate variability in growth response among replicates.

The findings demonstrate that all tested organisms were capable of growing on the durian-based agar formulations, indicating that the medium can support basic bacterial survival and multiplication. However, growth intensity remained consistently lower than the nutrient agar control, suggesting reduced efficiency in supporting optimal bacterial proliferation. Nutrient agar is considered a general-purpose medium because it contains balanced sources of peptones, beef extract, and other nutrients that readily support the growth of non-fastidious microorganisms (Odonkor & Ampofo, 2013). The consistently high growth intensity observed in the nutrient agar control (mean = 3.0) confirms its effectiveness as a standard culture medium.

The gradual increase in growth intensity with higher concentrations of durian formulation suggests a dose-dependent effect, where increased substrate concentration may enhance nutrient availability for microbial metabolism. Durian pulp contains carbohydrates, sugars, amino acids, vitamins, and minerals that may contribute to bacterial growth (Muhtadi & Ningrum, 2019). At lower concentrations (5% and 10%), the nutrient availability may have been insufficient to sustain vigorous bacterial multiplication, which explains the minimal growth observed among the isolates. Increasing the concentration to 15% likely enhanced nutrient accessibility, thereby improving bacterial growth. Among the organisms tested, *Staphylococcus aureus* exhibited the most notable increase in growth intensity, rising from minimal growth at 5% and 10% to moderate growth at 15%. This finding may indicate that *S. aureus* is more adaptable to nutrient-limited environments or better able to utilize the organic

components present in the durian substrate. According to Staphylococcus aureus physiology studies, the organism can metabolize a wide variety of carbon sources, enabling it to survive under different environmental conditions (Thomsen & Willerslev, 2014). In contrast, *Escherichia coli* and *Pseudomonas aeruginosa* showed only slight increases in growth intensity across the concentrations tested. The relatively lower growth response of *E. coli* may suggest that the durian-based medium lacked certain nitrogenous compounds or growth-promoting factors required for optimal proliferation. Meanwhile, *P. aeruginosa* demonstrated only modest enhancement despite its known metabolic versatility. This could indicate that some components of the durian formulation were not favorable for rapid growth or that inhibitory substances naturally present in durian may have affected bacterial multiplication. Durian fruit has been reported to contain phytochemicals and sulfur-containing compounds with potential antimicrobial activity, which may partially suppress bacterial growth (Khaksar et al., 2016).

Overall, the results suggest that while durian-based agar can support bacterial growth, its efficiency remains inferior to conventional nutrient agar. Nevertheless, the ability of all test organisms to grow indicates its potential as an alternative or supplementary culture medium, particularly in resource-limited settings where commercial media may be costly or less accessible. Further optimization of the formulation, such as supplementation with additional nitrogen sources or pH adjustment, may improve its growth-supporting capacity.

The physical appearance of *Staphylococcus aureus* (ATCC 25923) grown on 15% durian-based agar was observed and documented to assess its colony morphology and growth characteristics on the prepared medium. The representative appearance of the bacterial colonies is presented in Figure 2.

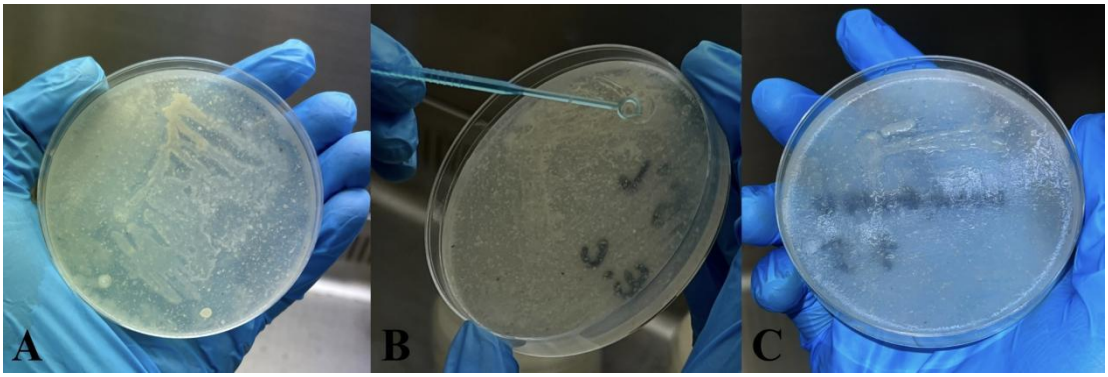

Figure 2. Physical appearance of *Staphylococcus aureus (*ATCC 25923) on 15% Durian-based agar

Colony Morphology and Pigmentation of Bacterial Isolates

Colony morphology and pigmentation of the bacterial isolates grown on durian-based agar and nutrient agar control were examined and recorded. Observations included colony size, shape, margin, elevation, and pigmentation differences across the different media.

Table 3. Colony Morphology and Pigmentation of Bacterial Isolates on Durian-Based Agar and Nutrient Agar

Bacteria	Medium	Color	Form	Margin	Remarks
S. aureus	Durian Agar	Creamy off white	Effused	Undefined	Hard to isolate
S. aureus	Nutrient Agar	Creamy	Circular	Entire	Distinct colonies
E. coli	Durian Agar	Creamy off white	Effused	Undefined	Spread growth
E. coli	Nutrient Agar	Off-white	Circular	Smooth	Well-defined
P. aeruginosa	Durian Agar	Creamy off white	Effused	Undefined	Confluent
P. aeruginosa	Nutrient Agar	Creamy	Circular	Defined	Pigmented

Table 3 shows the morphology of the bacterial colonies developed on durian-based agar which was effused and less distinct than colonies generated on nutrient agar. On the experimental medium, all the test organisms, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa,* produced creamy off-white colonies with unclear borders and confluent or spreading growth patterns. While the colonies

that grew on nutrient agar were clearly defined and circular and had morphological traits that were all similar to each other, showing that the control medium had greater isolation and growth differentiation. Extracts from durian have antimicrobial activity against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli*, factors in the reduction of colony definition seen in this study (Duazo et al., 2012). Studies on fruit waste-based media supports that the presence of phytochemicals ca change the growth of bacteria and produce less defined colony morphology compared to a standard nutrient agar (Senanayake, 2020). Similarly, culture media supplemented with plant extracts have been observed to promote confluent or diffused growth patterns by inhibiting bacterial cell division and colony organization (Calvo et al., 2014). The confluent growth of *Pseudomonas aeruginosa* on durian-based agar may indicate species-specific tolerance to plant-derived compounds. Gram-negative bacteria such as P. *aeruginosa* are known for their adaptive metabolic pathways and ability to survive in nutrient-variable environments, which may explain their continued growth despite medium modification (Berlanga, 2008).

Overall, the agar formulations based on durian were able to support the development of the test organisms such as Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa . However, the growth intensity was often lower than that of the

nutrient agar control, and the colony morphology was less distinct, particularly in colony isolation and structural clarity. Among the formulas, the 10% concentration was relatively better both in growth intensity and physical consistency. The results of this study indicate that durian pulp-based agar is a potential alternative culture medium but needs further modification to enhance its microbiological performance.

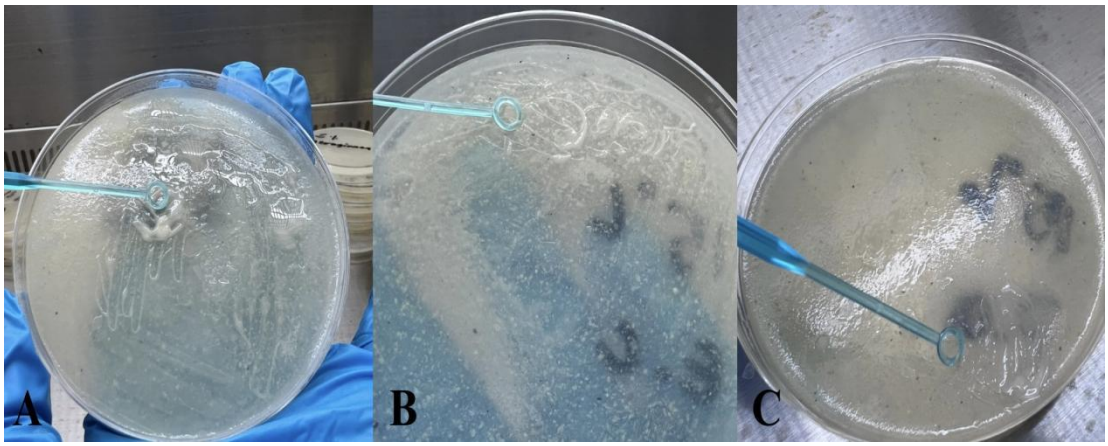

Figure 3. Colony Morphology of (A-C: *S. aureus, E. coli* and *P. aeruginosa*) on 15% concentration durian-based agar

CONCLUSION AND RECOMMENDATIONS

The study successfully demonstrated that durian (*Durio zibethinus*) pulp-based agar formulated at 5%, 10%, and 15% concentrations can support the growth of common pathogenic bacteria, including *Staphylococcus aureus (*ATCC 25923) , *Escherichia coli* (ATC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). However, bacterial growth intensity on the experimental media was consistently lower compared to nutrient agar, and colony morphology appeared effused, faint, and less distinct. Among the formulations, the 15% concentration showed relatively better performance in terms of growth intensity and physical consistency. Overall, the results indicate that while durian pulp-based agar has potential as a preliminary alternative culture medium, its current formulation is still inferior to standard nutrient agar in supporting optimal bacterial growth and differentiation.

Based on the findings, it is recommended that future studies further optimize the formulation of durian-based agar, particularly by adjusting nutrient composition and solidifying agents to improve bacterial growth performance and colony differentiation. Additional testing using a wider range of microorganisms and standardized conditions is also suggested to better evaluate its applicability as a culture medium. Moreover, it is recommended that future research consider alternative quantitative methods, as colony-forming unit (CFU) enumeration was not performed in this study due to confluent and effused colony growth, which prevented the isolation of discrete colonies necessary for accurate counting. Further refinement of growth assessment techniques, including more precise or automated measurement methods, may improve data accuracy and allow for more robust comparative analysis in future studies

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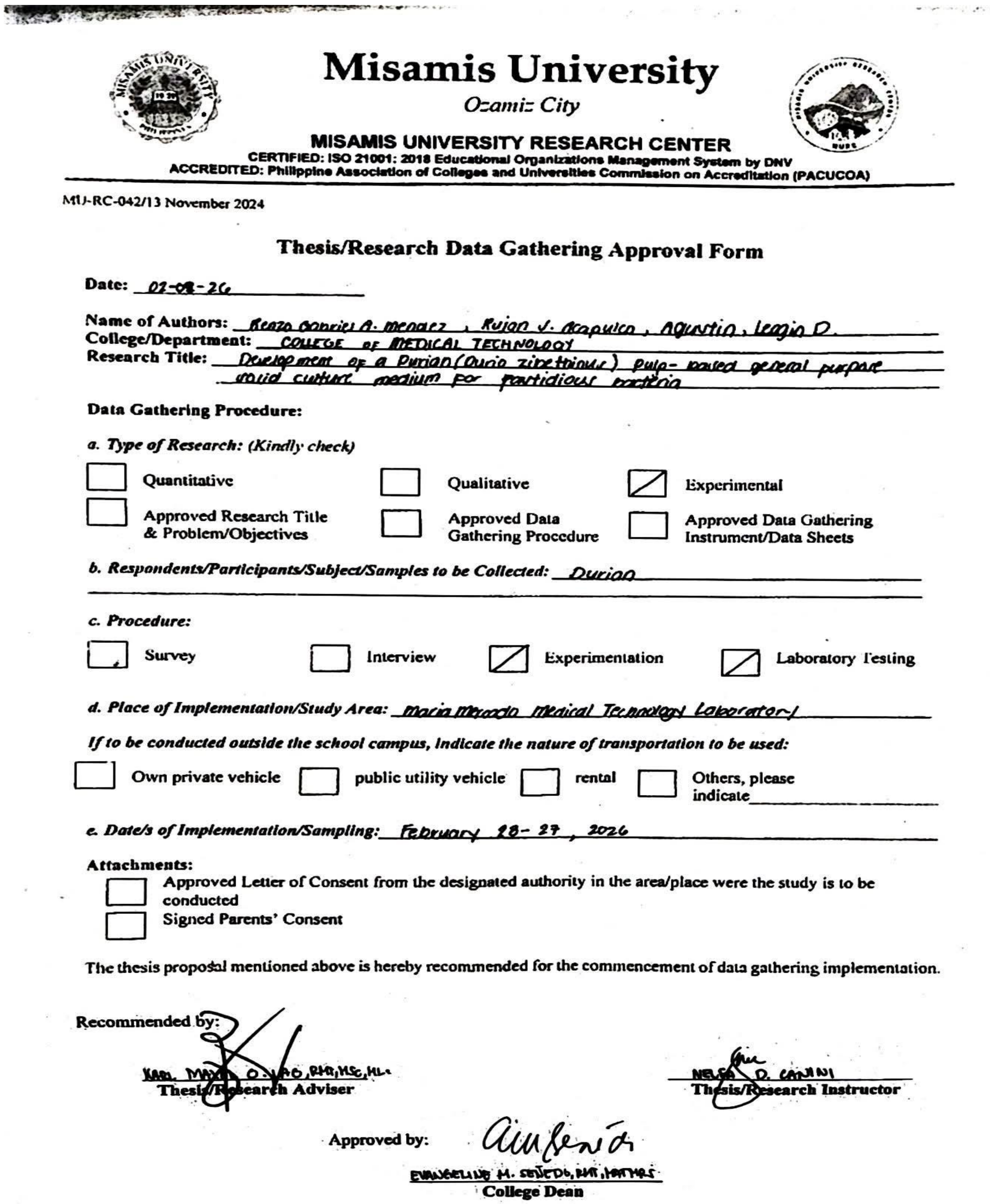
APPENDICES

A. Permission to Conduct the Study



Misamis University

Ozamiz 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)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: 02-08-26

Name of Authors: Renzo Gabriel A. Mendez, Ruján J. Acapulco, Agustin, Leagin D.
College/Department: COLLEGE of MEDICAL TECHNOLOGY
Research Title: Development of a Durian (Durio zibethinus) pulp-based general purpose solid culture medium for fastidious bacteria

Data Gathering Procedure:

a. Type of Research: (Kindly check)

- [ ] Quantitative
- [ ] Qualitative
- [x] Experimental
- [ ] Approved Research Title & Problem/Objectives
- [ ] Approved Data Gathering Procedure
- [ ] Approved Data Gathering Instrument/Data Sheets

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

c. Procedure:

- [ ] Survey
- [ ] Interview
- [x] Experimentation
- [x] Laboratory Testing

d. Place of Implementation/Study Area: Maria Mercado Medical Technology Laboratory

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 18-27, 2026

Attachments:

- [ ] Approved Letter of Consent from the designated authority in the area/place were 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:

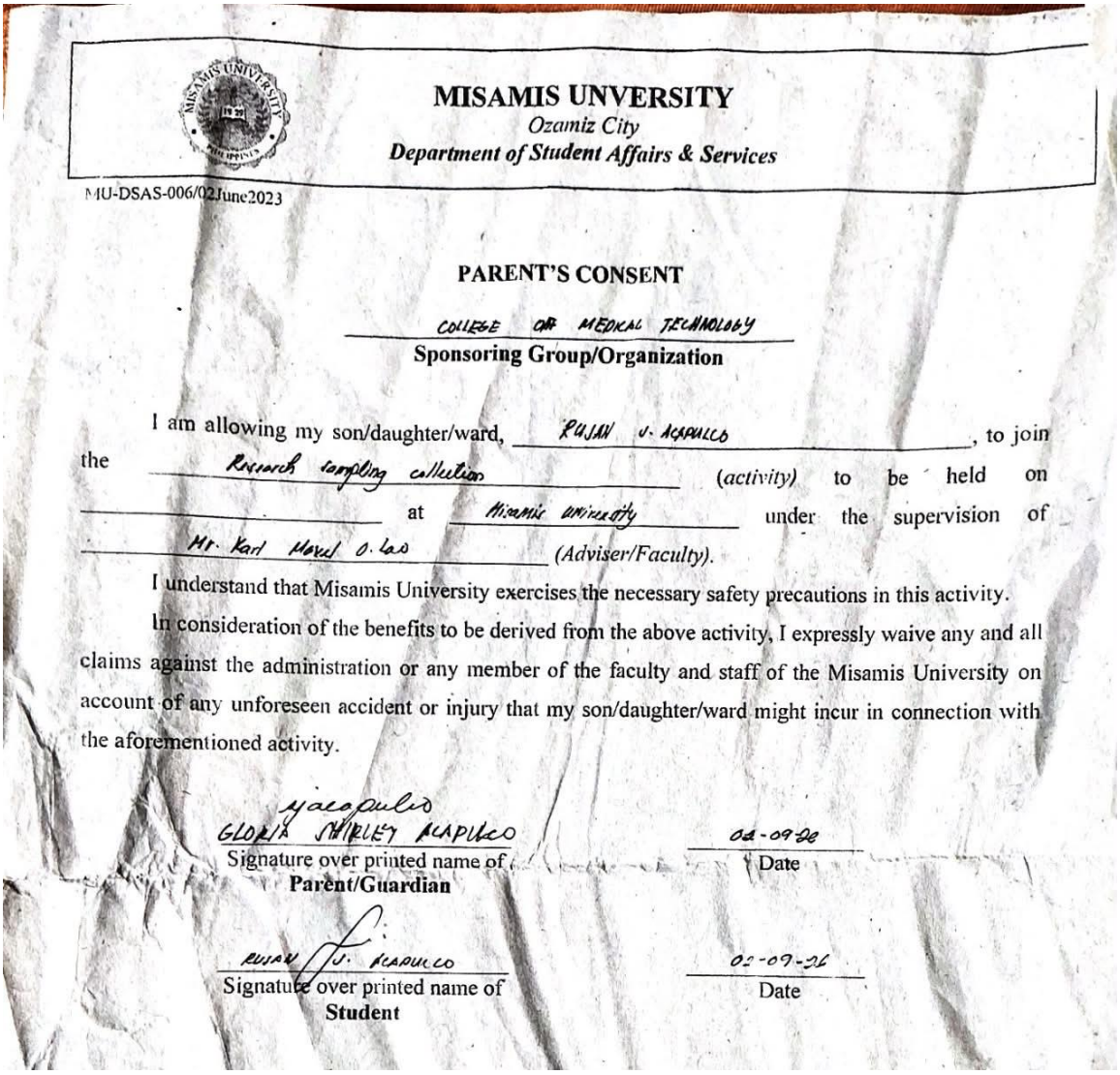
KARL MAXIM O. LAO, RMT, MSc, MLS
Thesis/Research Adviser

NELSA D. CANINI
Thesis/Research Instructor

Approved by:

EVANGELINE M. SENEDO, RMT, MAT-MS
College Dean

B. Approved Consent



MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

COLLEGE OF MEDICAL TECHNOLOGY
Sponsoring Group/Organization

I am allowing my son/daughter/ward, RUJAN V. ACAPULCO, to join the Research sampling collection *(activity)* to be held on _____ at Misamis University under the supervision of Mr. Karl Mervil O. Las *(Adviser/Faculty)*.

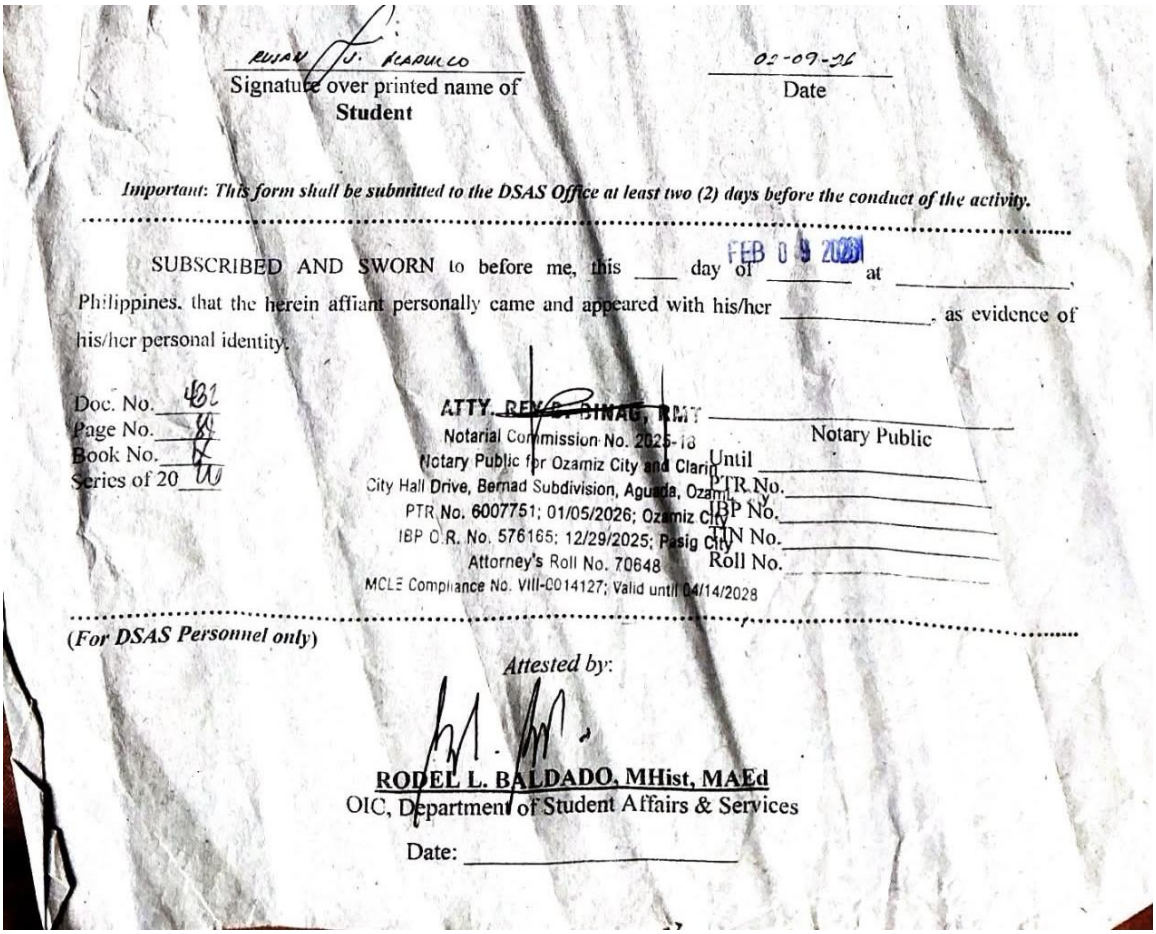
I understand that Misamis University exercises the necessary safety precautions in this activity.

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.

Acapulco
GLORIA SHIRLEY ACAPULCO — 02-09-26
Signature over printed name of **Parent/Guardian** — Date

RUJAN V. ACAPULCO — 02-09-26
Signature over printed name of **Student** — Date

APPENDIX B Cont'd



RUIAN J. ACAPULCO
Signature over printed name of **Student**

02-09-26
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 ____ day of FEB 09 2026 at _____, Philippines. that the herein affiant personally came and appeared with his/her _____. as evidence of his/her personal identity.

Doc. No. 461
Page No. 93
Book No. IX
Series of 2026

ATTY. REYES, BINAG, JR.
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

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

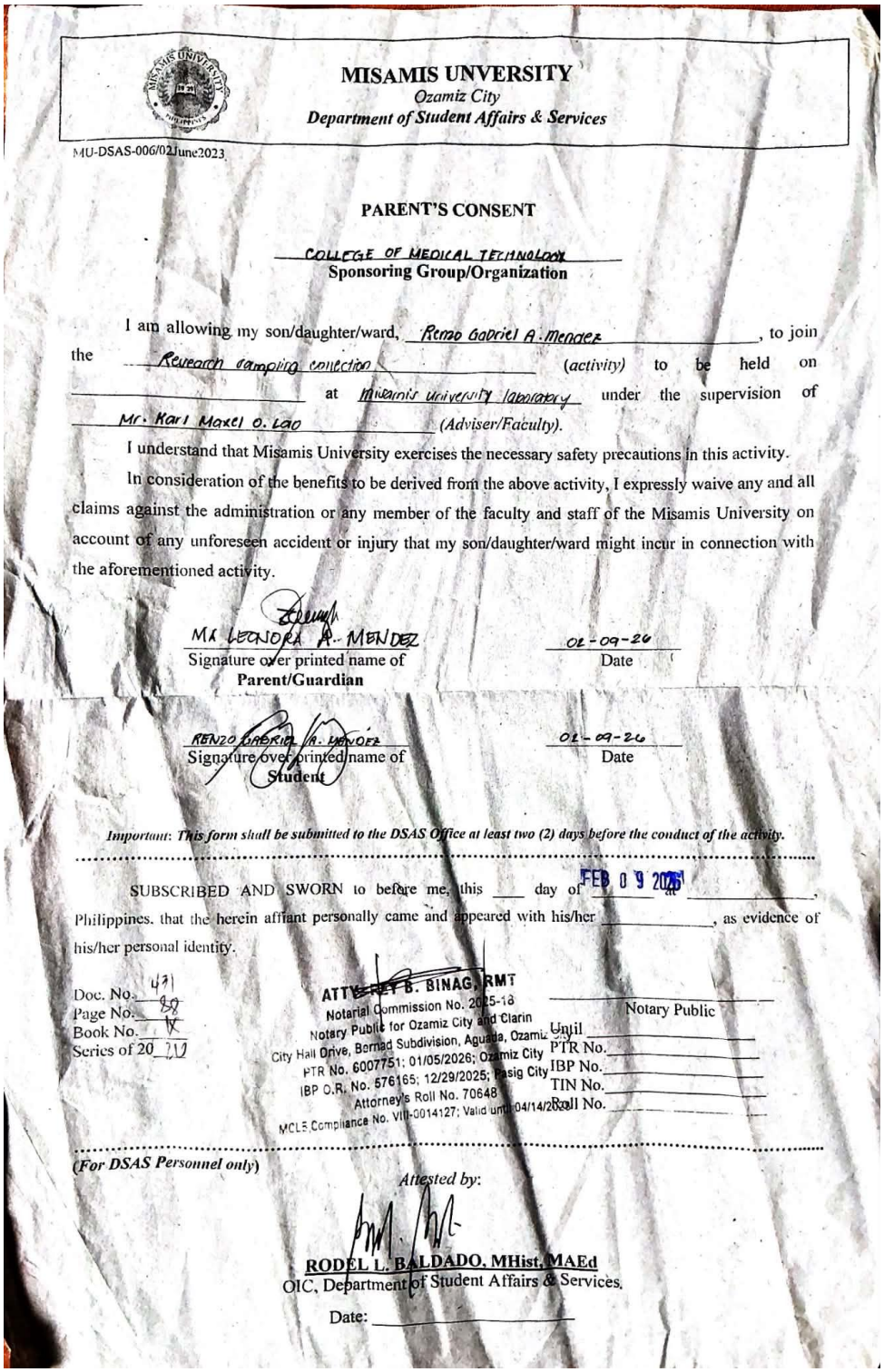
(***For DSAS Personnel only***)

Attested by:

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

Date: _____

APPENDIX B Cont'd



MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

COLLEGE OF MEDICAL TECHNOLOGY
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Renzo Gabriel A. Mendez, to join the Research sampling collection (*activity*) to be held on _____ at Misamis University laboratory under the supervision of Mr. Karl Maxel O. Lao (*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.

MA LEONORA A. MENDEZ — Signature over printed name of **Parent/Guardian** — 02-09-26 — Date

RENZO GABRIEL A. MENDEZ — Signature over printed name of **Student** — 02-09-26 — 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 ___ day of FEB 09 2026 at _____, Philippines. that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 471
Page No. 89
Book No. IV
Series of 20 26

ATTY. REY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-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: _____

C.Validity of Test Result

OBSERVATION NOTES

Title of the Study: DEVELOPMENT OF A DURIAN (Durio zibethinus) PULP-BASED GENERAL PURPOSE SOLID CULTURE MEDIUM FOR COMMON PATHOGENIC BACTERIA
Name of Researchers: Acapulco, Rujan J.; Agustin, Leogin D.; Mendez, Renzo Gabriel A.
Date Evaluated: May 11, 2026

Name of Statistician:
VIRGILIO A. EDULSA JR., MA-Math
PhD – Math (Candidate) – University of the Immaculate Conception, Davao City
virgilio.edulsa@deped.gov.ph
Kidapawan City NHS
Kidapawan City, North Cotabato

Dear researchers,

After reviewing the **Objectives of the Study**, **Data Analysis**, and the **Results and Discussion** sections of your research paper, the following are observed.

Statistical analysis and interpretation are **partially correct**, but there are several important technical and methodological issues that should be corrected or clarified.

A. Evaluation of Statistical Analysis
1. Alignment Between Objectives and Results
The objectives were:

1. Evaluate physical properties of durian-based agar
2. Assess and compare bacterial growth performance using qualitative growth intensity scoring
3. Compare colony morphology and pigmentation

The Results and Discussion generally addressed all three objectives:

- Table 1 → physical properties
- Table 2 → bacterial growth performance
- Table 3 → colony morphology

So, in terms of **coverage**, the **results align with the objectives**.

2. Major Statistical and Analytical Issues
A. The researchers claimed "descriptive statistical treatment" but did not actually present statistics properly
In the Data Analysis section, the study stated:
"Mean and standard deviation" were computed for each concentration

However, in the Results:

- Only **means** were shown
- No **standard deviations** presented
- No **tables of variability**
- No indication of replicate variation

This is a problem because the study had:

- 5 replicates per organism per concentration
- therefore, variability should have been reported.
- Without SD values, the analysis is incomplete.

APPENDIX C Cont'd

B. No actual comparative statistical test was performed
The study repeatedly used words like:

- "compare"
- "difference"
- "increase"
- "better performance"

These are test for significant difference.

But, in the study, there is **no inferential statistical test** that was conducted.
There was:

- no t-test
- no ANOVA
- no Kruskal-Wallis
- no p-values
- no significance testing

Yet the discussion interprets differences as meaningful but without concrete basis.

Example:
"15% concentration showed relatively better growth performance"
This may visually appear true, but statistically it was not tested.

Proper analysis should have included:
Since the data are ordinal qualitative scores:
Recommended tests (as applicable):

- Kruskal-Wallis test
- Mann-Whitney U test
- Friedman test (if repeated measures)

NOT parametric ANOVA unless assumptions are met.

C. The qualitative scoring scale is inconsistent
The legend states:

- 1.0 = no to faint growth
- 1.1–1.3 = slight growth
- 2.0 = moderate growth
- 3.0 = strong growth

But Table 2 contains:

- 1.4
- 2.4

These values are not defined in the scoring rubric.
This is a major methodological inconsistency.

Why this is problematic:
If scores are ordinal categories, averages like 2.4 may not be meaningful unless:

- multiple observers scored independently
- averages across replicates were computed

The interpretation scale must define ranges consistently.

D. Contradiction between Results and Conclusion
Table 2 shows:

S. aureus	5%	10%	15%
Mean	1.0	1.4	2.4

- This suggests 15% was clearly the best formulation for S. aureus.

APPENDIX C Cont'd

However, later the paper states:

- "Among the formulas, the 10% concentration was relatively better both in growth intensity and physical consistency."

This contradicts the actual data.

The conclusion later correctly says:

- "15% concentration showed relatively better performance"

So, the statement favoring 10% should likely be corrected to 15%.
This must be settled.

3. Specific Corrections Recommended
Statistical Improvements
Add:

- mean ± standard deviation
- replicate scores
- inferential statistical test

Recommended test:
For ordinal growth scores:

- Kruskal-Wallis test

If significant:

- Dunn's post hoc test

Correct Internal Contradiction
Replace:
"10% concentration was relatively better…"

with:
"15% concentration was relatively better…"
because the data support 15%.

Overall Evaluation
Statistical analysis: Partially correct but statistically incomplete.
The descriptive findings generally match the data presented, but the analysis has important methodological weaknesses:

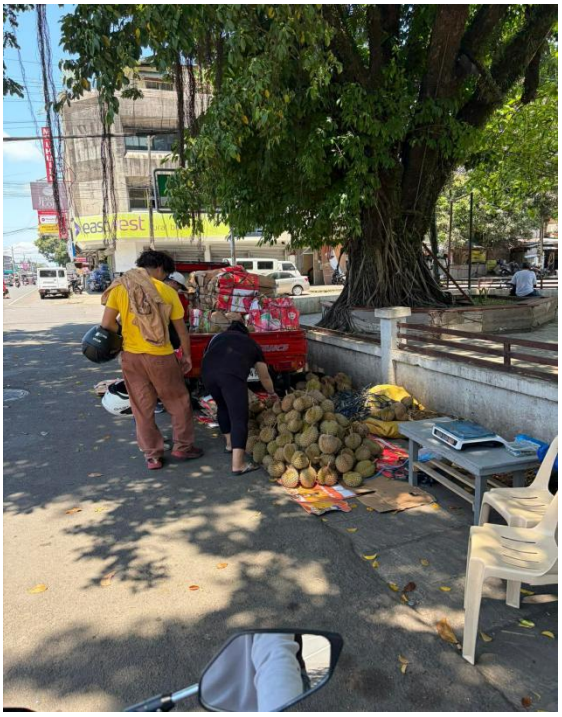
- missing standard deviations
- no inferential statistics
- inconsistent scoring scale
- unsupported comparative claims
- contradiction regarding best concentration

Conclusion:
The paper is acceptable as a **preliminary experimental study**, but the **statistical analysis needs strengthening before final submission or publication.**

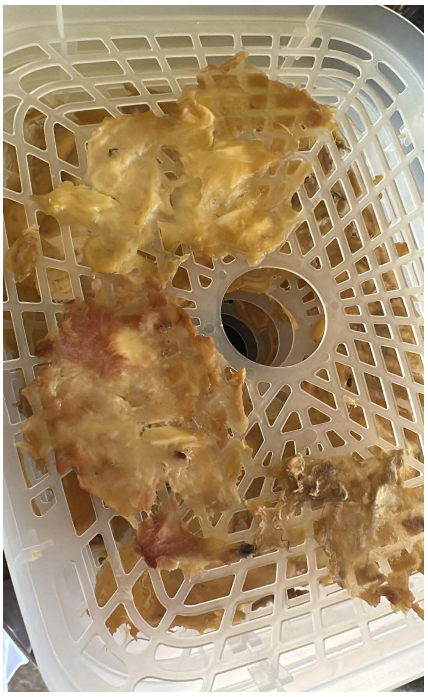
Checked and verified by:

VIRGILIO A. EDULSA JR., MA-Math
PhD – Math (Candidate)

D. Documentation



Appenfix D.1. Local Durian Supplier in Ozamiz City, Misamis Occidental

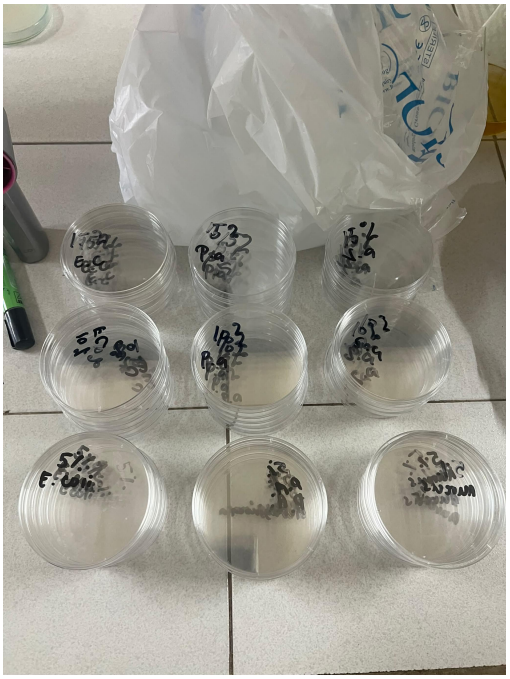


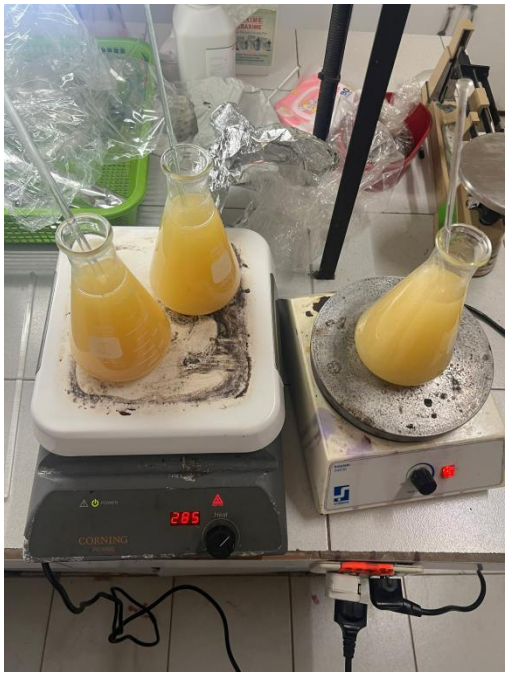
appendix D.2. Collection and Drying of Durian Pulp

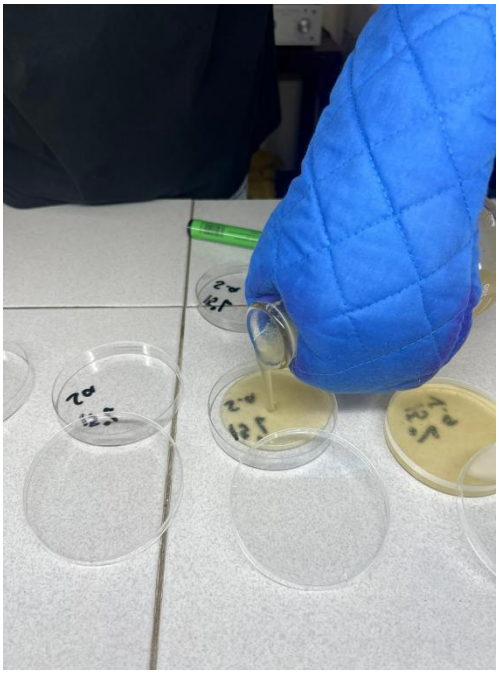
APPENDIX D Cont'd

Appendix D.4. Pulverized and Autoclaved Durian Sample

APPENDIX D Cont'd

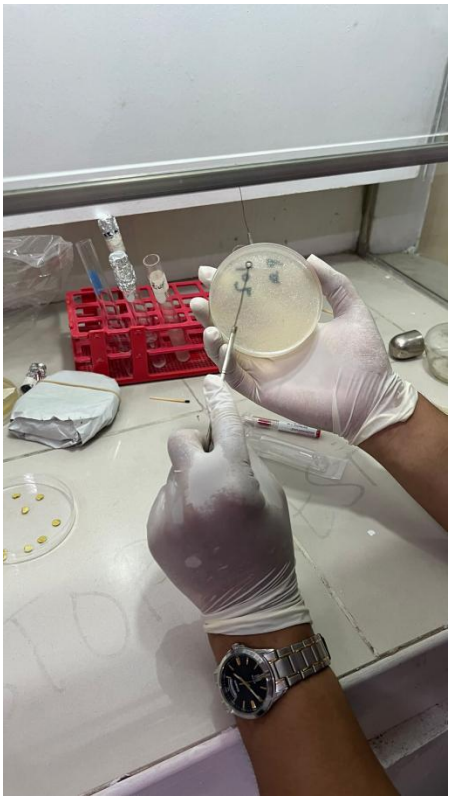






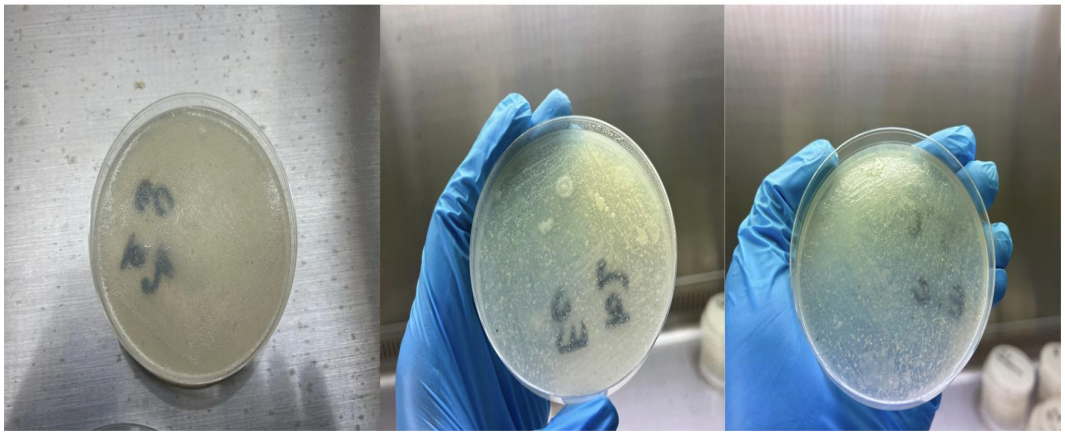
Appendix D.5. Preparation of Formulated Durian Agar

APPENDIX D Cont'd

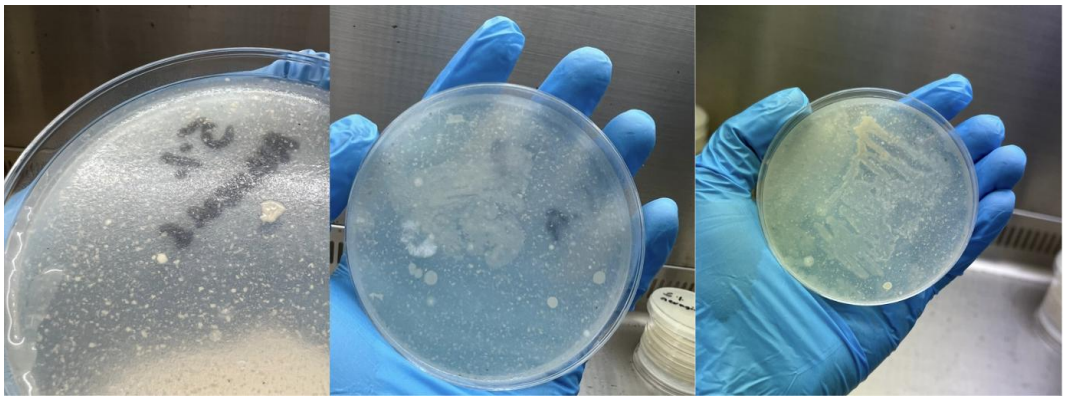


Appendix D.6. Inoculation of Bacteria on different concentrations

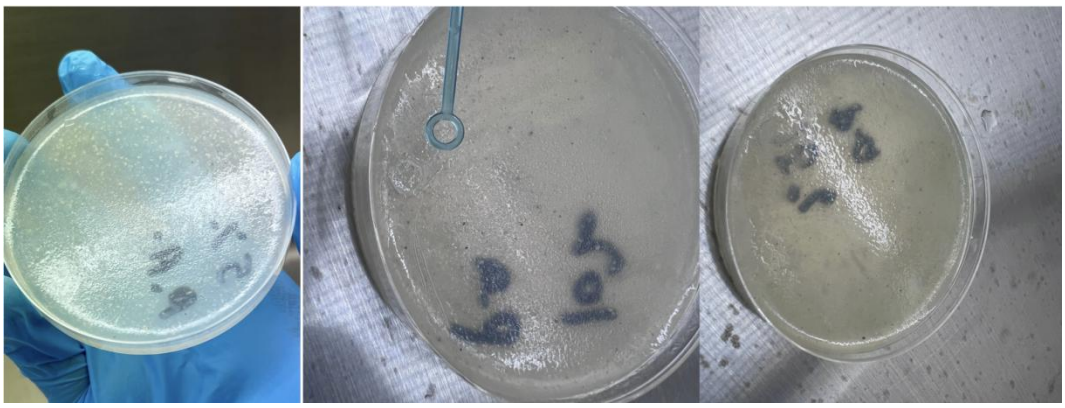
APPENDIX D Cont'd



Appendix D.7.*E.coli Strains a*t 5%-15% Concentration



Appendix D.8 *S. aureus*



Appendix D.9.*P. aeruginosa*

E. Raw Results

Bacteria	Medium	Concentration	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean
S. aureus	Durian Based Agar	5%	1	1	1	1	1	1
S. aureus	Durian Based Agar	10%	1.3	2	1.3	1.3	1.3	1.4
S. aureus	Durian Based Agar	15%	3	3	2	2	2	2.4
S. aureus	Nutrient Agar	Control	3	3	3	3	3	3
E. coli	Durian Based Agar	5%	1	1	1	1	1	1
E. coli	Durian Based Agar	10%	1.2	1.2	1.2	1.2	1.2	1.2
E. coli	Durian Based Agar	15%	1.3	1.3	1.3	1.3	1.3	1.3
E. coli	Nutrient Agar	Control	3	3	3	3	3	3
P. aeruginosa	Durian Based Agar	5%	1	1	1	1	1	1
P. aeruginosa	Durian Based Agar	10%	1.1	1.1	1.1	1.1	1.1	1.1
P. aeruginosa	Durian Based Agar	15%	1.2	1.2	1.2	1.2	1.2	1.2
P. aeruginosa	Nutrient Agar	Control	3	3	3	3	3	3

Appendix E.1. Mean Growth of Durian Based Agar Formulation

F. Grammarly and Turnitin Results

DEVELOPMENT OF A DURIAN (Durio zibethinus) PULP-BASED GENERAL PURPOSE SOLID CULTURE MEDIUM FOR COMMON PATHOGENIC BACTERIA

by Misamis University

General metrics

37,475	6,070	711	34 min 24 sec	1 hr 6 min
characters	words	sentences	reading time	speaking time

Score

99

This text scores better than 99% of all texts checked by Grammarly

Writing Issues

7	3	4
Issues left	Critical	Advanced

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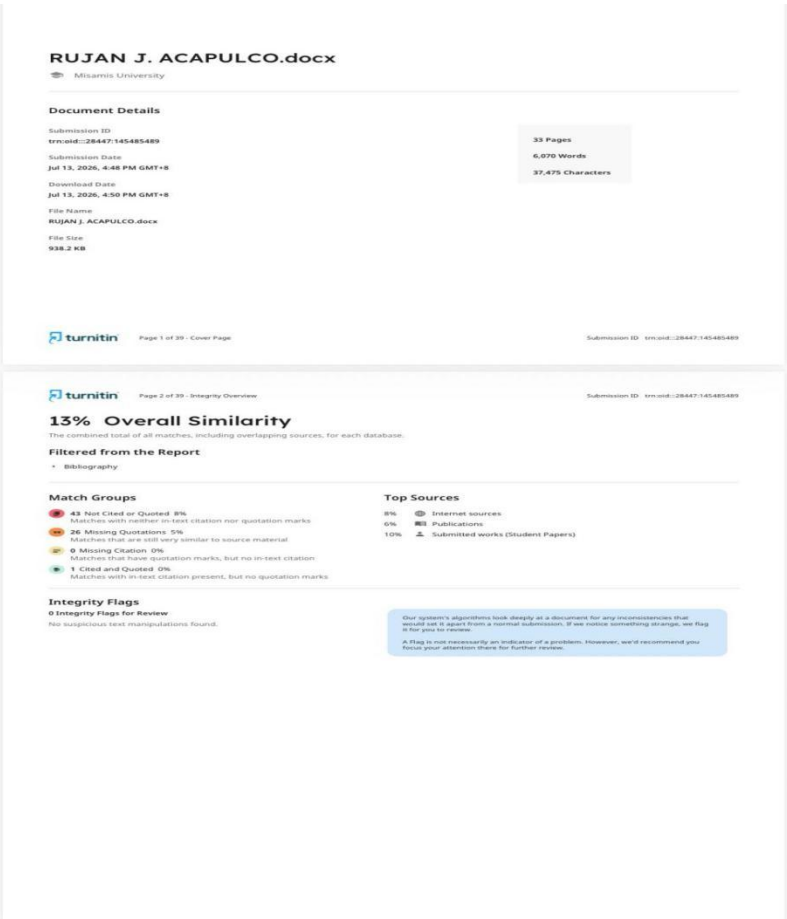

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