



**Preliminary Screening of the Antibacterial and Potential Synergistic
Effects of *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna
alata* (Acapulco) Leaf Extracts Against
Environmental Bacteria**

A Research Project submitted as partial
fulfillment of the requirements
in Practical Research 2

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Dedications

With utmost appreciation, we dedicate this research paper to Sir John Marie Malco, whose encouragement, perseverance, and priceless guidance have been instrumental to the triumph of this study. His passion in mentoring us, his support in times of difficulty, and his adherence to academic excellence have not only molded this research but also our personal development as emerging researchers. His confidence in what we can do has been a recurring source of inspiration, and to him we are deeply grateful.

We also dedicate this to every member of our research team, whose perseverance, resilience, and teamwork have made this study possible. Every difficulty we encountered, every debate we had, and every effort we exerted demonstrate the dedication we have towards scientific discovery. Without the diligence and perseverance of every researcher, this study would not have been achieved.

Additionally, a special dedication goes to someone whose encouragement and unwavering support reminded us to keep moving forward even in the face of doubt. Their words of motivation and belief in our abilities served as a silent strength throughout this journey.

Most importantly, we dedicate this research to the cause of scientific progression and to prospective researchers who will further seek antibacterial solutions using plants. Hopefully, this study will be



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the basis for making more discoveries to aid medicine, sustainability, and innovation.



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Abstract

The rise of antibiotic-resistant bacteria has increased the need for plant-based antibacterial alternatives. This study evaluates the antibacterial and wound-healing potential of *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna alata* (Acapulco) leaf extracts, individually and in combination, against environmental bacteria from soil, yogurt, and unwashed surfaces. Using the agar disk diffusion method, extracts at 25%, 50%, 75%, and 100% concentrations were tested for bacterial inhibition. Results revealed that *P. guajava* exhibited moderate antibacterial activity, particularly against Gram-positive bacteria, while *P. betle* and *S. alata* had minimal effects. The combined extracts showed synergistic antibacterial effects, with 75% concentration demonstrating the highest inhibition zones, particularly against Gram-negative bacteria from soil and unwashed surfaces. However, at 100%, inhibition zones decreased, suggesting a saturation effect or bacterial adaptation.

These findings highlight *P. guajava*'s potential as a natural antibacterial agent, reinforcing the importance of plant-based alternatives for infection control and wound healing. The study also supports the synergistic interaction of plant bioactives, suggesting that combined extracts may enhance antibacterial efficacy. While this research provides a foundation for natural antimicrobial applications in medicine, food safety, and pharmaceuticals, further studies should



optimize extraction methods, compound interactions, and clinical viability to maximize their therapeutic potential.



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Acknowledgement

We would like to express our sincerest appreciation to Sir John Marie Malco, our research adviser, for his consistent guidance, knowledge, and assistance during the duration of this study. His constructive feedback and dedication to academic excellence have been priceless in sharpening our research and ensuring its successful accomplishment.

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In addition, we recognize the collaborative work of our research team, whose commitment, persistence, and teamwork have been instrumental in taking this study to its completion. Each obstacle we faced was overcome with resolve, and it is through our collective passion for scientific research that this study was made possible.

Finally, we would like to express our sincere appreciation to our friends and families, whose constant support and encouragement have given us the strength and inspiration to finish this research. Their faith in us has been a source of inspiration throughout this process.

To all those individuals, in any role, who assisted in bringing this research to its conclusion, we offer our sincerest gratitude. We hope this



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study proves a valuable contribution to scientific endeavor and future study.



Introduction

The quest for other antibacterial agents has directed researchers to search for medicinal plants because of their abundance of bioactive compounds with antimicrobial and wound-healing activities. Some of these plants, such as *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna alata* (Acapulco), are highly recognized for their medicinal properties with flavonoids, tannins, and phenolic compounds that lead to bacterial inhibition and tissue healing. Though their separate antibacterial activities have been extensively described, there is limited information available regarding their combined synergistic action.

Environmental bacteria, which are usually present in soil, food, water, and high-touch surfaces, are hazardous in the form of food contamination, biofilm development, and infection. Unlike pathogenic strains, these bacteria are resilient in everyday environments and can become resistant to chemical disinfectants. Research points towards the contribution of built environments in microbial transmission and the importance of natural antibacterial solutions (National Academic Press, 2017) As bacterial resistance increases, medicinal plants offer a viable, sustainable option over synthetic antimicrobials.

Studies also indicate that when plant extracts are blended, there is an augmentation of their antibacterial activity. (Wirahmi et al., 2021) also observed that the combination of 75% *P. guajava* and 25% *P. betle* infusion had better antibacterial effects than the separate extracts. Also,



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(Igwe et al., 2023) proved that combining *S. alata*, *P. guajava*, and *A. wilkesians* produced bigger inhibition zones, indicating that there is synergy in antimicrobial interaction. Nevertheless, no previous research has assessed completely the synergistic antibacterial and wound-healing activity of *P. guajava*, *P. betle*, and *S. alata* in combination.

Aside from their antibacterial properties, these plants also have wound-healing potential. *P. betle* extract has been discovered to enhance cell proliferation and inhibit inflammation (Thi et al., 2021), while *P. guajava* can enhance wound closure (Ekom & Tamokou, 2018). *S. alata* also has bioactive compounds that lead to bacterial inhibition and tissue regeneration (Avila, 2025). Their collective wound-healing potential, however, has yet to be discovered.

This study seeks to determine the synergistic antibacterial action of *P. guajava*, *P. betle*, and *S. alata* on environmental bacteria isolated from soil, yogurt, and unwashed surfaces. Through the determination of their bacterial inhibition efficiency and wound-healing uses, the study hopes to be one step closer to the utilization of natural antibacterial formulations as a safer, more environmentally friendly option. Successful completion of this research would potentially be the basis of plant-based antibacterial products, which would be advantageous for clinical, pharmaceutical, and food safety sectors.



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Research Questions

This study aims to evaluate the antibacterial and wound-healing potentials of (*Psidium guajava*), (*Piper betle*), and (*Senna alata*) leaf extracts, both individually and in combination. Specifically, it seeks to answer the following questions:

1. What is the antibacterial activity of *P. guajava*, *P. betle*, and *S. alata* extracts when tested individually against environmental bacterial isolates from soil, yogurt, and unwashed surfaces, as measured by the zone of inhibition?
2. Does the combination of (*Psidium guajava*), (*Piper betle*), and (*Senna alata*) extracts exhibit a synergistic antibacterial effect compared to their individual applications, as determined by a statistically significant increase in the zone of inhibition?
3. What is the comparative antibacterial efficacy of the individual and combined plant extracts, and how do their inhibitory effects on bacterial growth differ in terms of zone of inhibition?
4. How do the antibacterial properties of the plant extracts correlate with their potential to enhance wound healing, based on their effectiveness in bacterial inhibition?
5. What are the practical implications of this study's findings?

Conceptual Framework

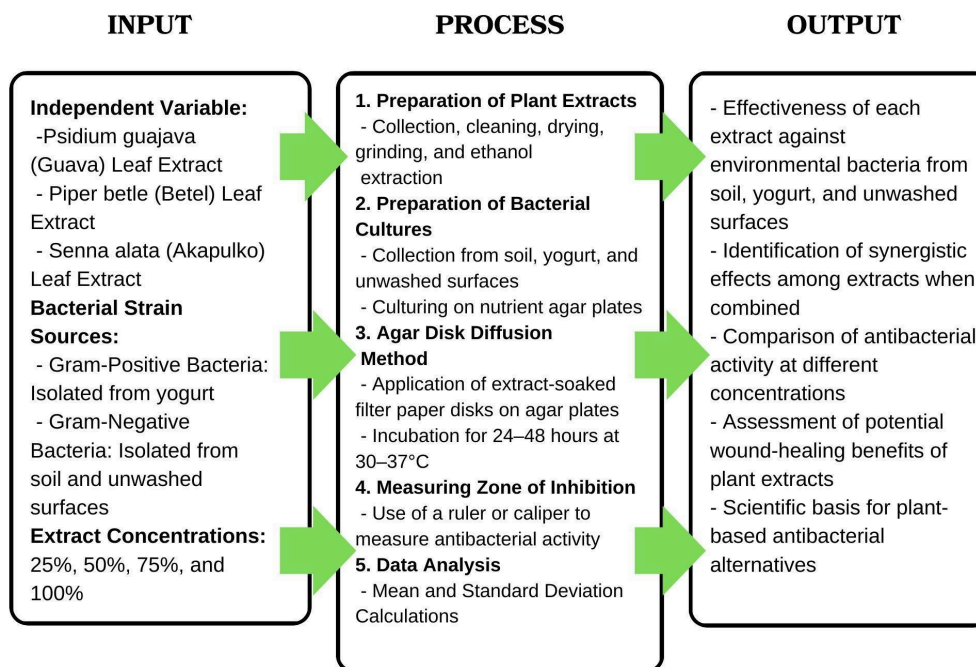


Figure 1.

The conceptual framework that will be used in this research study is the Input-Process-Output (IPO) model to determine the antibacterial properties of the different plant extracts. The input consists of Independent Variable: P. guajava (Guava), P. betle (Betel), and S. alata (Acapulco) leaf extracts, tested against environmental bacteria isolated from soil (Gram-negative), yogurt (Gram-positive), and unwashed surfaces (mixed bacteria). Therefore, these extracts are applied at varying concentrations of 25%, 50%, 75%, and 100%. The process starts with the preparation of plant extracts, which are collected, cleaned, dried, and ground before being extracted in ethanol. Followed by the preparation of



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bacterial cultures, the bacteria are collected from soil, yogurt or other unwashed surfaces, and then cultured on nutrient agar plates. In addition, agar disk diffusion method is used for applying the extract-soaked filter paper disks onto agar plates; then followed by incubation under 30-37 °C for 24-48 hours. The zones of inhibition are measured in mm using rulers or using calipers to measure its antibacterial activity. Data analysis is carried out by the statistical method, such as mean and standard deviation calculations. The output of this framework will give a comprehensive evaluation of plant extracts against environmental bacteria gathered from soil, yogurt, and unwashed surfaces. It will also identify the potential synergistic effects among the extracts when combined, enhancing their antibacterial properties. Therefore, this study will compare the antibacterial activity of the extracts at different concentrations to determine its efficacy, assessing the potential of these plant extracts to promote wound-healing and establishing a scientific basis for plant-based antibacterial activities.

Research Hypothesis

Null Hypotheses:

Ho1: There is no significant antibacterial activity of *P. guajava*, *P. betle*, and *S. alata* leaf extracts against environmental bacteria, as measured by the zone of inhibition



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Ho2: The combination of (*Psidium guajava*), (*Piper betle*), and (*Senna alata*) leaf extracts does not result in a statistically significant synergistic antibacterial effect compared to their individual applications, as determined by the zone of inhibition.

Ho3: There is no significant difference in the antibacterial efficacy between the individual and combined (*Psidium guajava*), (*Piper betle*), and (*Senna alata*) leaf extracts, as measured by the zone of inhibition.

Ho4: The antibacterial effects of the individual and combined plant extracts do not significantly correlate with their potential to promote wound healing, based on bacterial inhibition.

Ho5: The findings of this study have no practical implications for the development of plant-based antibacterial and wound-healing treatments.

Significance of the Study

Given the increasing antibiotic resistance and limitations with synthetic treatments, the use of medicinal plants as antibacterial agents and for wound healing has also gained momentum. This study involves the evaluation of the combined effects of *P. guajava* (Guava), *P. betle* (Betel), and *S. alata* (Acapulco) leaf extracts, intending to give scientific evidence of its synergistic effect. As the research explores the antibacterial and wound-healing potentials of these plants, it offers a promising pathway for developing alternative, sustainable therapies that can address the global rise of drug-resistant bacteria.



Moreover, this study is beneficial to the following:

Students. The study offers a prospective avenue for exploration into medicinal plant research and allows the further delving of knowledge into alternative therapies. The insights this study will uncover for the practical applications of plant-based antimicrobial agents will be beneficial, especially to health sciences and biotechnology students pursuing further studies and careers in these fields.

Research Teachers. Educators will find this study useful because it showcases the need for interdisciplinary research and the discovery of natural products in medicine. It could be used as a template for future research projects, challenging students to think critically and analytically. Moreover, it will contribute to the existing academic dialogue

on the development of sustainable alternatives to conventional antibiotic treatments.

School Administrators. School administrators can base their appeals on the inclusion of environmental sustainability as part of their healthcare education offerings. Encouraging plant-based therapies can tie in with wider initiatives around environmentally friendly and healthy practices within an educational setting to further solidify the commitment towards scientific innovation from the institution itself.



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Future Researchers. Future researchers are able to build on the background knowledge and methodology developed in this research as a foundation to continue to explore the synergistic action of plant extracts. With the results and methodology developed here, researchers are able to continue to explore the potential of other mixtures of plants, paving the way for the creation of new, safe, and effective natural medicines.

Scope and Delimitation

This study assesses the antibacterial activity of leaf extracts of *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna alata* (Acapulco) against naturally occurring environmental bacteria. For bio-safety and researcher safety, this study does not use laboratory-cultivated pathogenic bacteria. Instead, naturally occurring environmental bacteria from soil, yogurt, and unwashed surfaces are used as safe alternatives for antimicrobial testing. Bacterial samples are instead isolated from soil, yogurt, and unwashed surfaces, which offer a safe yet scientifically acceptable substitute for antimicrobial testing.

The research will aim to find out the concentration-dependent antibacterial activity of the plant extracts, varying concentrations (25%, 50%, 75%, and 100%) will be examined to check if higher concentrations give more bacterial inhibition. Zone of inhibition and bacterial growth inhibition on nutrient agar plates will be used to determine the efficacy of these extracts. But identification of bacteria by Gram staining or



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biochemical reactions will not be carried out. Instead, growth of bacteria will be seen visually with colony formation, and there should be a negative control to verify the outcome.

This study only involves in vitro antibacterial assays and does not include long-term antibacterial action, resistance by bacteria, toxicity tests, or human medicine clinical applications. Additionally, it does not evaluate the economic viability, marketability, or consumer acceptability of such plant extracts as antibacterial compounds. Although the use of environmental bacteria permits an initial screening for antibacterial activity, the findings may not directly extend to medical or pharmaceutical uses. It is suggested that future work should confirm these observations by challenging the plant extracts against clinically relevant bacterial strains in bio-safety-controlled laboratory conditions.

Definition of Terms

Antibiotic Resistance- Antibiotic resistance develops when bacteria evolve to the point that antibiotics no longer kill or inhibit their growth. As a result, bacterial infections are particularly difficult to cure. (Professional, 2024)

Antifungal Activities- Antifungals are substances that kill or prevent the growth of the fungi or spores that cause infection. (Antifungals, 2025)



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Antimicrobial Therapeutic- Treatments or drugs which inhibit the growth or kill of microbes such as bacteria, fungi, or viruses.

Bioactive Compounds- Bioactive chemicals are chemical substances present in small amounts in foods such as fruits, vegetables, nuts, whole grains, and oils that influence physiological activities in the human body. (Teodoro, 2019)

Gram-negative Bacteria- These bacteria fail to retain the stain with crystal violet, are resistant to certain antibiotics and, due to an outer membrane, it's hard to be treated. (Stoakes, 2018)

Gram-Positive Bacteria- They are a sort of bacteria characterized by retaining the crystal violet dye used in Gram staining, cell wall is comparatively thick, and are often even more sensitive than Gram-negative towards many drugs or antibiotics. (Steward, 2023)

Minimum Inhibitory Concentration- The smallest concentration of an antibacterial agent that could inhibit the growth of any microorganism. (Krochmal & Wicher, 2021)

Phytochemicals- Natural chemicals produced by plants, including flavonoids, tannins, and phenols, that may have medicinal properties, including antibacterial and anti-inflammatory effects. (Huang & Edirisinghe, 2016)



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Piper betle- Commonly called “betel leaf,” it is a vine belonging to the Piperaceae family, and betel leaves have a mildly stimulant effect when chewed.(Shipa & Mohamed, 2022)

Psidium guajava- Commonly known as “Guava,” it is a tropical fruit-bearing plant belonging to the family Myrtaceae. (Kumar et. al., 2021)

Senna alata- Also known as “Cassia alata,” Senna alata is a plant species in the legume family, and it has traditionally been used in herbal medicine to treat skin conditions like ringworm and other fungal infections.(Habtemariam, 2019)

Zone of Inhibition- The area surrounding an antimicrobial agent where bacteria cannot grow. It is used to determine the effectiveness of the agent. (Basak & Banerjee, 2022)



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Methodology

Research Design

This study employs an experimental research design, specifically the agar disk diffusion assay, to evaluate the antibacterial and potential wound-healing properties of *Psidium guajava*, *Piper betle*, and *Senna alata* leaf extracts. The test organisms include environmental Gram-positive bacteria (Lactobacillus species from yogurt) and Gram-negative bacteria (from soil and unwashed surfaces). To ensure scientific accuracy and controlled experimentation, key variables such as plant extract concentrations (25%, 50%, 75%, and 100%) and bacterial strain types were carefully standardized. Positive (Betadine) and negative (sterile water) controls were also included to validate antibacterial activity.

Respondents and Sampling Technique

Since this study does not involve human subjects, environmental bacteria will serve as the experimental subjects. The selection of bacterial cultures will focus on strains relevant to wound infections and antimicrobial resistance.

Bacterial Strains

Instead of using pathogenic bacteria, environmental bacteria will be collected from soil, yogurt, and unwashed surfaces to serve as

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Gram-positive and Gram-negative bacterial sources. These isolates will be used to evaluate the antibacterial activity of the plant extracts.

Sampling Technique

Inclusion Criteria:

- Gram-positive bacteria: Lactobacillus species isolated from yogurt
- Gram-negative bacteria: Environmental bacteria isolated from soil and unwashed surfaces

Exclusion Criteria:

- Bacterial strains that are difficult to cultivate or identify using standard techniques
- Extract concentrations outside the 25%–100% range

Experimental Design to Screen the Antimicrobial Activity on Plant Extracts

Group	Bacteria Strains	Plant Extract (S)	Concentrations	Numbers of Trials
1.	Bacteria from Soil	P. guajava	25%,50%, 75%,100%	3
2.	Bacteria from Soil	P. betle	25%,50%, 75%,100%	3



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3.	Bacteria from Soil	S. alata	25%,50%, 75%,100%	3
4.	Bacterial from Soil	Combination of Guava,Betel, Acapulco	25%,50%, 75%,100%	3
5.	Bacteria from Yogurt	P. guajava	25%,50%, 75%,100%	3
6.	Bacteria from Yogurt	P. betle	25%,50%, 75%,100%	3
7.	Bacteria from Yogurt	S. alata	25%,50%, 75%,100%	3
8.	Bacteria from Yogurt	Combination of Guava,Betel, Acapulco	25%,50%, 75%,100%	3

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9.	Bacteria from Surface	<i>P. guajava</i>	25%,50%, 75%,100%	3
10.	Bacteria from Surface	<i>P. betle</i>	25%,50%, 75%,100%	3
11.	Bacteria from Surface	<i>S. alata</i>	25%,50%, 75%,100%	3
12.	Bacteria from Surface	Combination of Guava,Betel, Acapulco	25%,50%, 75%,100%	3

Table 1.

As presented in Table 1, the experimental groups will evaluate the leaf extracts' antibacterial activity against Gram-positive bacteria (yogurt) and Gram-negative bacteria (soil and unwashed surfaces). These plants are chosen for their previously established antimicrobial activity such as *Psidium guajava* with bacteriostatic and bactericidal activity against a number of bacteria, *Piper betle* that has exhibited excellent



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antibacterial activity owing to its high content of phenols and *Senna alata* with its antimicrobial and wound-healing activity. *Piper betle*, however, has demonstrated very strong bactericidal activity against a range of bacteria. Every extract of the plant will be assayed for four different concentrations (25%, 50%, 75%, and 100%), and their combination will also be assayed to find if they have synergistic antibacterial effects. Combined extracts will also be assayed to find if there is a synergistic effect to say that the combined extracts are more potent at inhibiting bacterial growth compared to individual extracts. In order to establish the accuracy and consistency, three repetitions of every treatment condition will be performed. The zone of inhibition around the filter paper discs treated with extracts will be used as the quantitative assessment of antibacterial activity. The larger inhibition zone will result in a higher antibacterial activity. In order to provide accuracy and consistency for the results, each experimental condition will be carried out in triplicate. The results will yield important information on the possible therapeutic uses of these plant extracts as natural antimicrobial agents.

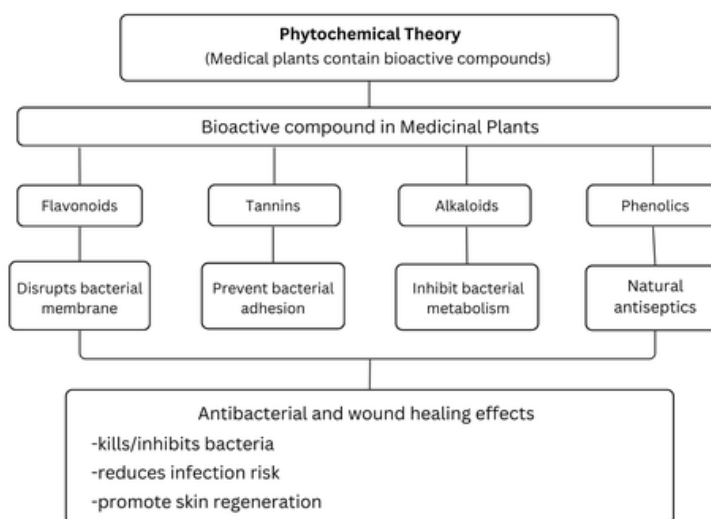
Theoretical Framework

This study is based on three main theories: the Phytochemical Theory, Synergistic Antibacterial Theory, and Wound-Healing Theory. These are the premises for the knowledge that *Psidium guajava* (Guava),

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Piper betle (Betel), and *Senna alata* (Acapulco) leaf extracts, when used synergistically, exhibit antibacterial and wound-healing activities. The Phytochemical Theory assumes how bioactive compounds from medicinal plants play a role in antimicrobial activity and tissue repair. The Synergistic Antibacterial Theory explains how the combination of several plant extracts is more effective in antibacterial action than their individual applications. Finally, the Wound-Healing Theory explains how bio-active compounds help in tissue repair and inhibit infection. All these theories together give a scientific explanation for studying the combined antibacterial and wound-healing activity of these medicinal plants.

Phytochemical Theory

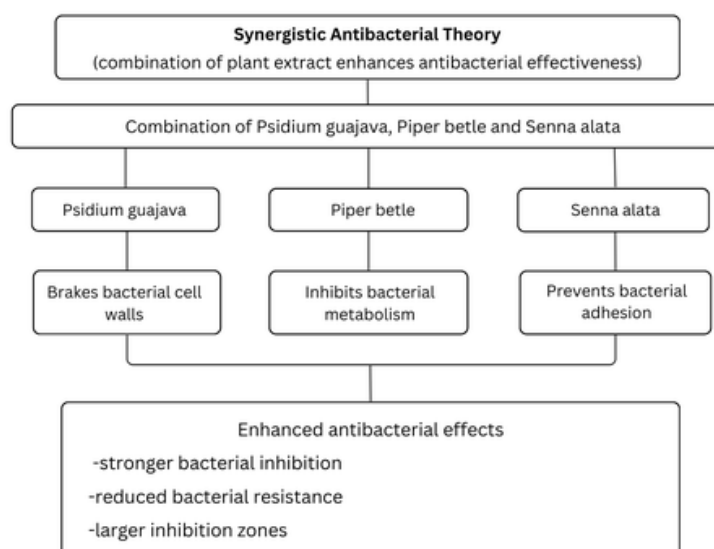


The Phytochemical Theory confirms that medicinal plants have secondary metabolites like flavonoids, tannins, alkaloids, and phenolic compounds that play a pivotal role in antibacterial and wound-healing

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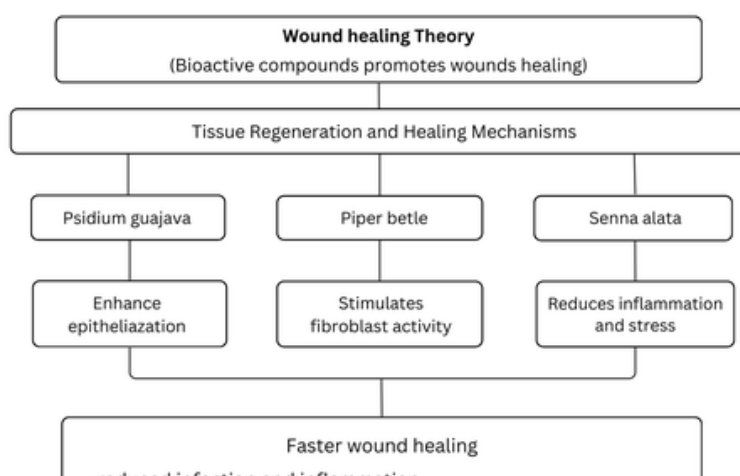
activities (Gutiérrez et al., 2008). Such bioactive compounds act as a natural defense system against microbial infections. *Psidium guajava* is a wealth of flavonoids that induce bacterial membrane disruption and antimicrobial growth inhibition (Biswas et al., 2013). Piper betle is rich in alkaloids, which disrupt bacterial metabolism and cause cell death of bacteria (Subashkumar et al., 2013). *Senna alata* has phenolic compounds and tannins that are natural antiseptics, denaturing proteins of bacteria and inhibiting bacterial adhesion and proliferation (Avila, 2025). Combining these bio-active compounds gives them a wide spectrum of antibacterial activity. Igwe et al. (2023) proved the application of composite medicinal plant extracts exhibited more intensified bacterial inhibition in comparison to a single-extract application, consequently reinforcing further examination of the combined synergistic influence of *P. guajava*, *P. betle*, and *S. alata*.

Synergistic Antibacterial Theory



Synergistic Antibacterial Theory validates that the synergism of several plant extracts increases antibacterial activity as they act upon various bacterial pathways simultaneously. Whereas each plant extract has antibacterial activity when alone, their effect together creates wider inhibition zones with less bacterial resistance. *Psidium guajava* disrupts bacterial cell wall stability, which leads to cellular content leakage and structural damage (Biswas et al., 2013). Piper betle inhibits the metabolism of bacteria, disrupting fundamental processes that promote bacterial survival and growth (Mahfuzul Hoque et al., 2011). *Senna alata* inhibits the adhesion of bacteria and the formation of biofilms, which are the major means of bacterial resistance (Donkor, 2016). The effects complement each other when the three plant extracts are applied in combination, thus the antibacterial activity is improved. Igwe et al. (2023) reaffirmed that multi-plant formulations exhibited significantly more potent antimicrobial activity than single-plant treatments, substantiating their use for effective bacterial inhibition.

Wound-Healing Theory





The Wound-Healing Theory explains the way bio-active compounds enhance tissue regeneration, diminish inflammation, and inhibit bacterial infections, all of which are vital parameters in wound healing (Thi et al., 2021). This theory highlights that reducing bacterial load and inflammation is the best setting for skin cell regeneration. *Piper betle* was found to increase fibroblast activity, causing more collagen synthesis and faster wound closure (Thi et al., 2021). *Senna alata* has anti-inflammatory activity, which inhibits swelling and oxidative stress—two of the key factors that slow down the healing process (Donkor, 2016). *Psidium guajava* is rich in flavonoids and tannins, which have been reported to increase epithelialization and granulation tissue formation, leading to quicker skin repair (Ekom & Tamokou, 2018). The simultaneous application of these plant extracts is likely to provide a wound-healing environment by suppressing infection, inflammation, and promoting tissue regeneration.

Experimental Procedure

A. Preparation of Plant Extracts



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1. Plant Sample Collection and Cleaning

Fresh leaves of *Senna alata* (Acapulco), *Piper betle* (Betel), and *Psidium guajava* (Guava) will be picked from a fresh, uncontaminated source. Fresh leaves of *Psidium guajava*, *Piper betle*, and *Senna alata* were collected from uncontaminated sources. To ensure sterility, the leaves were rinsed with sterile distilled water and air-dried at room temperature (25–30°C) for 3–5 days under shade to prevent photodegradation of bioactive compounds.

2. Extraction of Bio-active Compounds

The powdered leaves were subjected to maceration in 70% ethanol for 48 hours to extract bioactive antibacterial compounds. Ethanol (70%) was chosen as the solvent due to its ability to extract both polar and non-polar phytochemicals, including flavonoids, tannins, and phenolic acids, which contribute to antibacterial activity (Govindasamy et al., 2022).

3. Filtration and Concentration of Extracts

After 48 hours, the extract will be filtered with filter paper to remove the liquid extract from solid leaf particles. The filtered liquid will be transferred to a rotary evaporator or left in an open sterile dish and left at room temperature to enable evaporation of the ethanol, leaving only the concentrated plant extract behind. Such concentrated extract is



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regarded as 100% strength and will be utilized as the base for future dilutions.

4. Dilution of Extracts to Different Concentrations

The 100% concentrated plant extract will be diluted with sterile distilled water to obtain lower concentrations (75%, 50%, 25%) using the formula:

$$C_1V_1=C_2V_2$$

where C = concentration and V = volume of the extract solution

B. Preparation of Bacterial Cultures

1. Preparation of Bacterial Cultures

Since the objective of this research is to analyze the antibacterial activities of *Psidium guajava*, *Piper betle*, and *Senna alata* extracts instead of the isolation of individual bacterial species, environmental bacteria shall serve as a scientifically valid and safe alternative to the pathogenic strains proved in the laboratory. Rather than cultivating *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, bacteria will be acquired from naturally occurring sources to replicate actual microbial exposure while maintaining research safety. Sources of bacteria include:

Soil - Contains a diverse range of bacteria, including Gram-negative species.

Yogurt – Contains *Lactobacillus*, a common Gram-positive bacterium.



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Unwashed Surfaces- door handles, keyboards, or skin Represents bacteria commonly found in daily environments.

To verify bacterial viability and guard against experimental invalidity, the following will be done:

Visual Verification of Bacterial Growth

- The inoculated nutrient agar plates shall be incubated at a suitable temperature of 25–30°C for 24–48 hours.
- Visible presence of well-defined bacterial colonies (round, irregular, white, yellowish, or slimy) will indicate successful growth of bacteria.

Justification for Using Environmental Bacteria

The use of environmental bacteria instead of pathogenic strains aligns with biosafety guidelines (Biosafety Level 1 standards), ensuring a safe yet scientifically relevant assessment of antibacterial activity. Since real-world bacterial exposure includes both beneficial and opportunistic pathogens, this study models practical microbial interactions while maintaining ethical laboratory safety protocols (WHO, 2021). Although this study does not do species-level bacterial identification (Gram staining or biochemical testing), it adheres to routine antimicrobial testing protocols in that it visually validates the presence of bacterial growth prior to using plant extracts.



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2. Preparation of Nutrient Agar Plates

Nutrient agar plates will be prepared to culture the bacteria with the following procedures:

1. Dissolve 23g of nutrient agar powder in 1 liter of distilled water in a beaker and stir well.
2. Sterilize the nutrient agar solution using an autoclave at 121°C for 15 minutes under 15 psi pressure
3. Let the agar slightly cool, and pour 15–20 mL into each of the sterile Petri dishes.
4. Keep the plates alone for 30–45 minutes to permit the agar to solidify.
5. Store plates upside down in a fridge if not immediately used to avoid moisture accumulation.

3. Collection and Streaking of Bacteria

1. Moisten a sterile cotton swab with sterile distilled water.
2. Collect bacteria from one of the selected sources:
 - Dip the swab in a diluted soil solution (1g soil in 10mL sterile water).
 - Dip the swab in yogurt or a probiotic drink.
 - Swab a door handle, keyboard, or unwashed hands.
3. Gently streak the swab across the agar plate in a zig-zag motion to distribute bacteria evenly.
4. Label the plate with the sample source and date.



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5. Incubate the plates at 30–37°C for 24–48 hours to allow bacterial growth.

C. Testing Antibacterial Activity Using Agar Disk Diffusion

1. Preparing the Filter Paper Disks

1. Cut small 6 mm circular disks from filter paper.
2. Prepare four separate sets of filter paper disks, each soaked in one of the plant extract concentrations (25%, 50%, 75%, 100%).
3. Prepare two control disks:
 - Positive Control: Soaked in an antibiotic solution (amoxicillin) or antiseptic (Betadine).
 - Negative Control: Soaked in sterile distilled water to verify that results are due to the plant extracts.
4. Allow all disks to absorb their respective solutions for a few minutes before placement.

2. Assigning Experimental Groups

The bacterial strains will be divided into 12 groups, each testing different plant extracts:

Group	Bacterial Strain Source	Plant Extract(s)	Concentrations	Number of Trials



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1	Soil Bacteria	Psidium guajava	25%, 50%, 75%, 100%	3
2	Soil Bacteria	Piper betle	25%, 50%, 75%, 100%	3
3	Soil Bacteria	Senna Alata	25%, 50%, 75%, 100%	3
4	Soil Bacteria	Combination of (P. guajava, S. Alata, P. betle)	25%, 50%, 75%, 100%	3
5	Yogurt Bacteria	Psidium guajava	25%, 50%, 75%, 100%	3
6	Yogurt Bacteria	Piper betle	25%, 50%, 75%, 100%	3
7	Yogurt Bacteria	Senna Alata	25%, 50%, 75%, 100%	3

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8	Yogurt Bacteria	Combination of (P. guajava, S. Alata, P. betle)	25%, 50%, 75%, 100%	3
9	Surface Bacteria	Psidium guajava	25%, 50%, 75%, 100%	3
10	Surface Bacteria	Piper betle	25%, 50%, 75%, 100%	3
11	Surface Bacteria	Senna Alata	25%, 50%, 75%, 100%	3
12	Surface Bacteria	Combination of (P. guajava, S. Alata, P. betle)	25%, 50%, 75%, 100%	3

Table 2.

3. Placement of the Disks on Bacteria-Grown Agar Plates

1. Open the incubated bacterial plates.

2. Using sterile forceps, filter paper disks (12.7 diameter, Filter paper) were carefully placed on the inoculated agar plates:



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- Four disks containing different extract concentrations (25%, 50%, 75%, 100%).
- One Positive Control Disk (Betadine).
- One Negative Control Disk (Sterile distilled water).

To maintain accuracy and prevent interference, disks were placed equidistantly from each other to avoid overlapping inhibition zones (CLSI, 2021)

3. Ensure disks are evenly spaced and not touching.

4. Incubation and Observation

1. Close the Petri dishes and incubate at 30–37°C for 24–48 hours.
2. After incubation, observe and measure zones of inhibition (clear areas where bacteria did not grow).

5. Measuring and Recording Results

1. Use a ruler or caliper to measure the diameter of inhibition zones (in mm).
2. Compare the inhibition zones for different extract concentrations.
3. Compare plant extracts to the positive and negative controls.

D. Disposal and Sterilization

1. Used agar plates will be soaked in bleach or 70% alcohol before disposal.
2. Glassware and reusable materials will be disinfected.



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3. Leftover plant material will be discarded in biodegradable waste bins.

Data Collection Procedure

1. Recording Zone of Inhibition

After incubation, inhibition zones will be measured in millimeters using. A standard ruler (± 1 mm precision) was used to measure the diameter of inhibition zones (in mm). Measurements were taken from the outer edge of the clear zone, ensuring consistent and reproducible readings. To validate findings, inhibition zones were measured three times per sample, and the mean diameter was calculated to reduce measurement variability. Compared across different extract concentrations to determine dose-dependent antibacterial effects. Analyzed against the positive and negative controls to assess the relative antibacterial efficacy of the plant extracts.

2. Organizing Data for Analysis

- Each extract concentration (25%, 50%, 75%, 100%) will have three trials.
- The mean and standard deviation of the inhibition zones will be calculated for each treatment group.
- Results will be grouped based on bacterial strain and extract type (individual vs. combination).

3. Ensuring Accuracy & Reliability

- Each sample will be tested in triplicate to ensure consistency.



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-Measurements will be taken by at least two researchers to minimize human error.

-Photographic documentation of inhibition zones may be taken for reference.

4. Data Verification & Entry

-Raw data will be checked for inconsistencies before statistical analysis.

-A spreadsheet or laboratory logbook will be used for systematic data entry.

Group Assignments

Gram-Positive Bacteria: Yogurt Isolates (*Lactobacillus* and related species)

Group 1: *Psidium guajava* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 2: *Piper betle* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 3: *Senna alata* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 4: Combination of *Psidium guajava*, *Piper betle*, and *Senna alata* extracts (25%, 50%, 75%, 100%) – 3 trials each

Gram-Negative Bacteria: Soil Isolates

Group 5: *Psidium guajava* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 6: *Piper betle* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 7: *Senna alata* extract (25%, 50%, 75%, 100%) – 3 trials each



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Group 8: Combination of *Psidium guajava*, *Piper betle*, and *Senna alata* extracts (25%, 50%, 75%, 100%) – 3 trials each

Gram-Negative Bacteria: Unwashed Surface

Group 9: *Psidium guajava* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 10: *Piper betle* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 11: *Senna alata* extract (25%, 50%, 75%, 100%) – 3 trials each

Group 12: Combination of *Psidium guajava*, *Piper betle*, and *Senna alata* extracts (25%, 50%, 75%, 100%) – 3 trials each.

Data Analysis

Data Scoring

The antibacterial efficacy shall be expressed in the diameter of inhibition zones around the filter paper discs. Inhibition zones with a larger diameter indicate stronger antibacterial activity.

Results and Discussion

This section presents the findings of the study, including bacterial culture growth, the disk diffusion assay, and the antibacterial activity of guava, betel, and acapulco leaf extracts against different bacterial sources (yogurt, soil, and unwashed surface bacteria). The zone of inhibition (ZOI) was measured in millimeters (mm) to determine the antibacterial efficacy of each extract.

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Bacterial Culture Growth After Three Days

To ensure sufficient bacterial growth before testing the antibacterial properties of plant extracts, bacterial cultures were incubated for three days. The images below show the colony formation of yogurt bacteria, soil bacteria, and unwashed surface bacteria on agar plates.

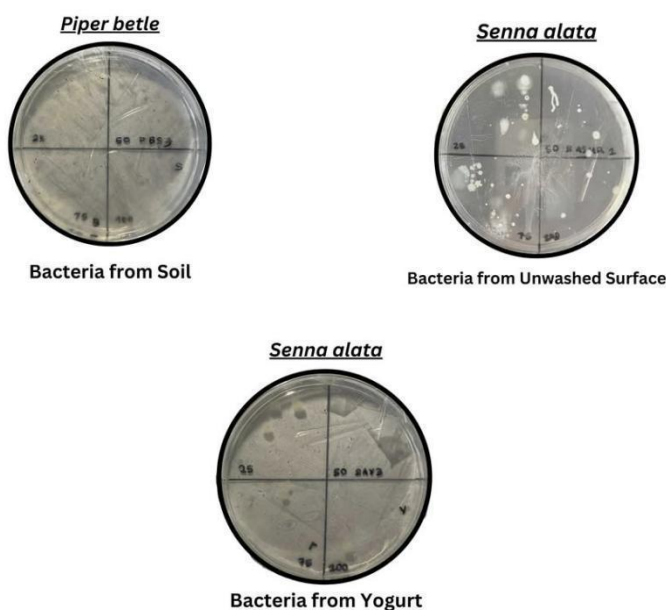


Figure 1.

Testing Disk Diffusion on Different Plant Extracts and Bacteria

The disk diffusion method was performed by placing filter paper disks soaked in guava, betel, and acapulco extracts on bacterial cultures. The setup ensured even distribution of the extracts and allowed for clear observation of antibacterial effects based on the presence of inhibition zones around the disks.

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Senna alata



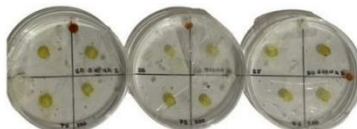
Replicates 1-3 (Soil)

Senna alata



Replicates 1-3 (Yogurt)

Senna alata



Replicates 1-3 (Unwashed Surface)

Psidium guajava



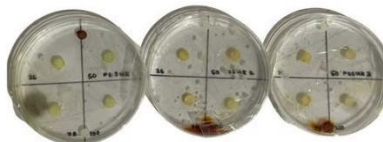
Replicates 1-3 (Soil)

Psidium guajava



Replicates 1-3 (Yogurt)

Psidium guajava



Replicates 1-3 (Unwashed Surface)

Piper betle



Replicates 1-3 (Soil)

Piper betle

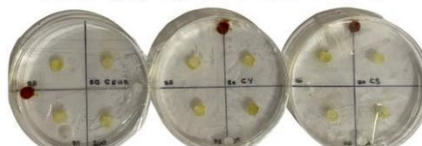


Replicates 1-3 (Unwashed Surface)

Piper betle



(COMBINED EXTRACTS)
Psidium guajava, Piper betle, Senna alata



Replicate 1
Unwashed Surface

Replicate 2
Yogurt

Replicate 3
Soil

Results of the Zone of Inhibition

After incubation, the antibacterial activity of each extract was assessed by measuring the zone of inhibition (mm) around the disks. The following tables present the measured inhibition zones for each extract against different bacterial sources.

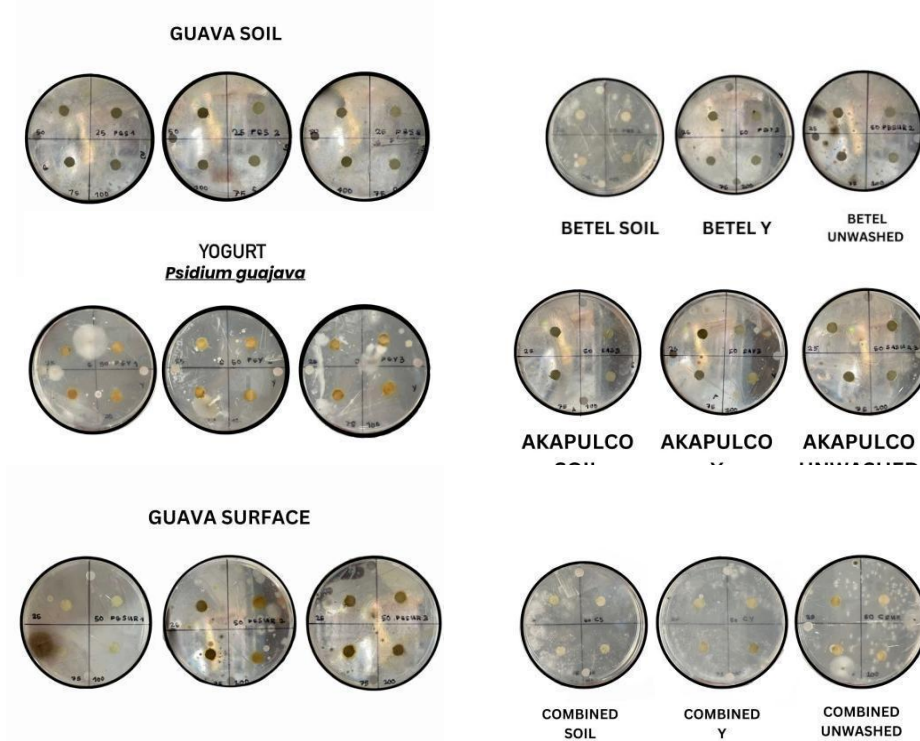


Figure 3.

GUAVA AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation



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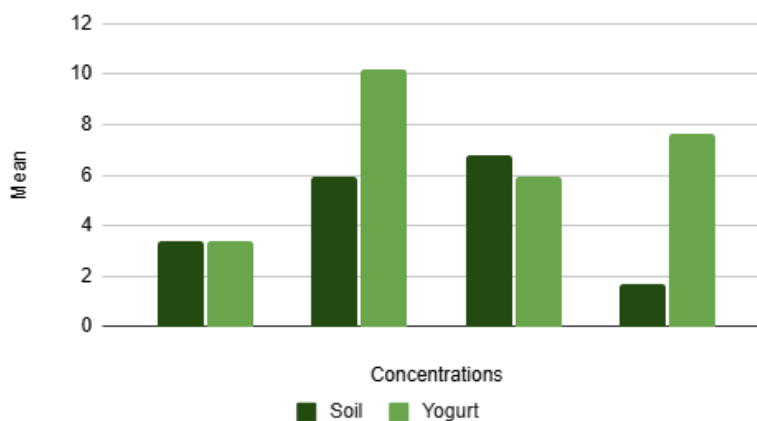
25%	0mm	0mm	10.16m m	3.38	5.86	Minimal to no inhibitio n
50%	10.16 mm	10.16m m	0mm	10.16	1.79	Strong inhibitio n
75%	7.62m m	10.16m m	10.16m m	5.92	5.28	Moderate inhibitio n
100%	5.08m m	7.62m m	10.16m m	7.62	2.54	Strong inhibitio n
GUAVA AGAINST SOIL BACTERIA						
Concentratio ns	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standar d Deviatio n	Interpret ation
25%	0mm	10.16	0mm	3.38	5.86	No to minimal inhibitio n
50%	0mm	7.62m m	10.16m m	5.92	5.28	Mild inhibitio n
75%	0mm	10.16m m	10.16m m	6.77	5.86	Moderate inhibitio n
100%	0mm	5.08m m	0mm	1.69	2.93	No to minimal

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						inhibition
GUAVA AGAINST UNWASHED SURFACE BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone

Table 3.

Effect of *P. guajava* Extract on Environmental Bacteria



The experiment evaluated the antibacterial activity of *Psidium guajava* (guava) leaf extract against soil bacteria, yogurt bacteria, and



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surface bacteria that are not washed with concentrations of 25%, 50%, 75%, and 100% based on the zone of inhibition (mm) as an effectiveness measure.

At 25% concentration, guava extract showed low or no inhibition (0 mm–10.16 mm) against soil bacteria, suggesting that a minimal amount of bioactive compounds is insufficient for bacterial inhibition. Moderate inhibition was observed at 50% and 75% (7.62 mm–10.16 mm), confirming dose-dependent activity. However, 100% concentration had variable effects (0 mm–5.08 mm), indicating that higher concentrations do not always lead to stronger antibacterial effects, potentially due to compound saturation or bacterial adaptation (Ngene et al., 2019).

For yogurt bacteria (Gram-positive), guava extract at 50% and 100% concentrations showed strong inhibition (7.62 mm–10.16 mm), while 75% had moderate inhibition. This aligns with (Alam et al., 2023), who reported that guava leaf essential oil is highly effective against Gram-positive bacteria, likely due to its flavonoid and tannin content, which can disrupt bacterial cell walls. More research is required to establish if guava extract selectively inhibits pathogenic bacteria or has an effect on beneficial probiotic strains as well.

For surface bacteria that were not washed, inhibition was not noted at any of the concentrations (0 mm), which is evidence of strong resistance of the bacteria, which was probably due to the existence of gram-negative organisms. This confirms (Govindasamy et al., 2022), who



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reported that some bacteria show structural resistance against plant antimicrobials and that increased formulations or higher levels are needed.

Overall, guava extract exhibited strong antibacterial activity against Gram-positive yogurt bacteria, moderate effects against soil bacteria, and no inhibition against unwashed surface bacteria (likely Gram-negative species). The findings support its selective antibacterial activity, particularly against Gram-positive bacteria, but highlight its limitations against Gram-negative bacteria due to their protective outer membrane (Govindasamy et al., 2022). Though higher levels (50%-100%) typically augmented inhibition, the 100% level was not always best, indicating saturation or adaptation of the compounds. These results validate earlier research affirming guava leaf extract's promise as a natural antibacterial, specifically against gram-positive bacteria, but indicating its limitations against gram-negative bacteria. Future studies should aim at phytochemical profiling, alternative extraction practices, and further testing with bacteria to maximize its antimicrobial efficacy.

BETEL AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone



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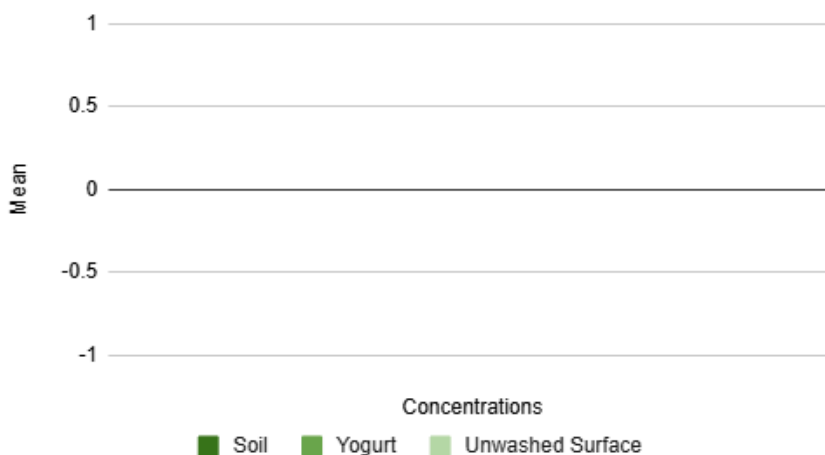
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
BETEL AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
BETEL AGAINST UNWASHED SURFACE BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation

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25%	0mm	0mm	0mm	0	0	No inhibiti on zone
50%	0mm	0mm	0mm	0	0	No inhibiti on zone
75%	0mm	0mm	0mm	0	0	No inhibiti on zone
100%	0mm	0mm	0mm	0	0	No inhibiti on zone

Table 4.

Effect of *P. betel* Extract on Environmental Bacteria



Betel extract was tested at 25%, 50%, 75%, and 100% concentrations, but no inhibition was observed across all bacterial strains. This suggests that the extraction method used did not successfully isolate active antibacterial compounds or that *Piper betle* lacks significant antibacterial properties in the tested conditions. No



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inhibition was observed in all the trials, and it was concluded that betel extract, as prepared in this experiment, was not showing antibacterial activity against the tested bacterial strains.

In case of unwashed surface bacteria, the lack of inhibition indicates that gram-negative species are present, which have an outer membrane conferring resistance against plant-derived antimicrobials. This agrees with the findings of (Zamri et al., 2023), who observed that although *P. betle* is traditionally used for medicinal applications, scientific evidence about its antibacterial activity is not yet sufficient and emphasizes the requirement for more research. Likewise, yogurt bacteria, which are mostly gram-positive lactic acid bacteria, were not inhibited, indicating that betel extract does not contain bioactive compounds that can inhibit probiotic or food-related bacteria. Soil bacteria were also not inhibited at any concentration, reinforcing the extract's ineffectiveness as an antibacterial agent. These findings suggest that betel extract, as it stands, is not very effective as an antibacterial agent.

These results differ from (Fazal et al., 2014) who reported strong antibacterial activity in *Piper betle* extracts. The discrepancy suggests that extraction methods and solvent types significantly impact its effectiveness. Future studies should explore ethanol-based extraction or different bacterial strains to determine if *P. betle* exhibits antibacterial potential under optimized conditions. The difference suggests that the extraction method, concentration, or type of bacterial strains tested

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could make a difference in its efficacy. Research in the future can look into other methods of extraction, like ethanol extraction, to improve its antimicrobial activity. In addition, phytochemical profiling also needs to be done in order to ascertain if betel extract has bioactive compounds with antibacterial activity. Extension of the analysis to clinically pertinent bacterial strains can also give a more accurate picture of its medicinally useful potential. In summary, betel extract did not exhibit any antibacterial effect in this study, highlighting the necessity of further research to ascertain if it possesses antimicrobial potential under optimized conditions.

ACAPULCO AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
ACAPULCO AGAINST SOIL BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone

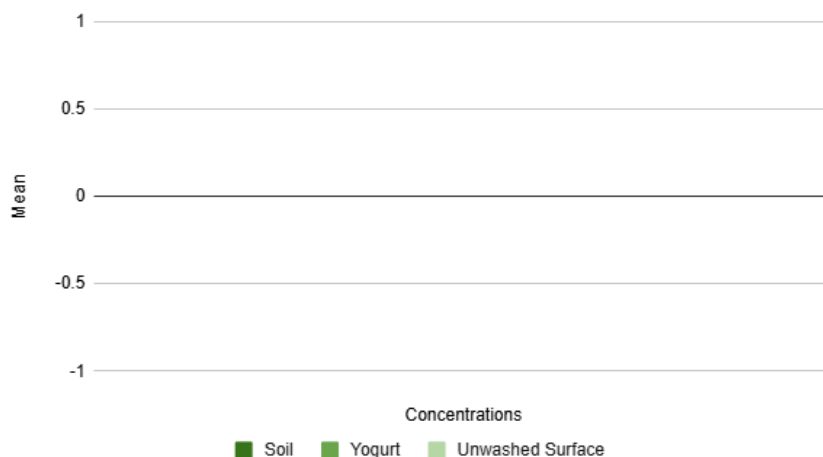


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50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
ACAPULCO AGAINST UNWASHED SURFACE BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone

Table 5.

Effect of *S. alata* Extract on Environmental Bacteria





The antibacterial effect of *Senna alata* (Acapulco) extract was tested on unwashed surface bacteria, yogurt bacteria, and soil bacteria in concentrations of 25%, 50%, 75%, and 100%. The findings indicated no inhibition in all the experiments, which revealed that acapulco extract was not found to have antibacterial activity against the tested strains of bacteria.

The lack of inhibition against unwashed surface bacteria suggests that Gram-negative bacteria were present, which possess an outer lipopolysaccharide (LPS) layer, reducing permeability to plant-derived antimicrobials (Nikaido, 2003). In the same manner, yogurt bacteria, which are mostly gram-positive lactic acid bacteria, were also not affected, suggesting that acapulco extract does not contain the bioactive compounds required to inhibit food- or probiotic-associated bacteria. Neither did the soil bacteria get inhibited with any concentration, further validating the extract's ineffectiveness as an antibacterial agent.

These results contrast with previous findings, such as (Sagnia et al., 2014) and (Tababa et al., 2020), which demonstrated that *Senna alata* extracts exhibit antifungal properties. The lack of antibacterial activity in this study suggests that *S. alata*'s antimicrobial effects are more specific to fungal rather than bacterial pathogens. Their research, however, concentrated on antifungal activity and not antibacterial action,

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so the difference in results could be due to this. The absence of antibacterial activity found in this study implies that *S. alata*'s antimicrobial activity could be specific to fungal pathogens and not bacterial strains. These findings show that acapulco extract, as it is presented now, lacks significant antibacterial activity.

Alternative extraction methods, like ethanol extraction, should be investigated in future studies to ascertain whether a different solvent increases its bioactive capacity. Also, phytochemical profiling must be done to detect any antibacterial compounds that can be found at low levels. Broadening testing to cover clinically relevant bacterial strains might further illuminate its possible applications. Overall, *S. alata* extract did not show antibacterial activity at any level of concentration, reinforcing the necessity of further research on its particular antimicrobial properties and best extraction protocols.

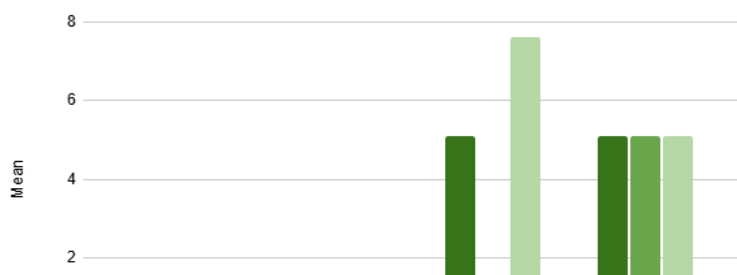
COMBINED EXTRACTS AGAINST SOIL BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	7.62mm	7.62mm	5.08	0	No to moderate inhibition

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100%	5.08mm	5.08mm	5.08mm	5.08	0	Mild inhibition
COMBINED EXTRACTS AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
50%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
75%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
100%	5.08mm	5.08mm	5.08mm	5.08 mm	0mm	Moderate inhibition
COMBINED EXTRACTS AGAINST UNWASHED SURFACE						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	7.62mm	7.62mm	7.62mm	7.62	0	Moderate inhibition
100%	5.08mm	5.08mm	5.08mm	5.08	0	Moderate inhibition

Table 6.

Effect of Combined Extract on Environmental Bacteria





The antibacterial activity of the combined extracts of *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna alata* (Acapulco) was assessed against yogurt bacteria, unwashed surface bacteria, and bacteria from soil at 25%, 50%, 75%, and 100% concentrations. The findings indicated no inhibition against bacteria in yogurt at 25%, 50%, and 75% and weak inhibition (5.08 mm) at 100%, reflecting poor antibacterial activity against gram-positive bacteria.

For unwashed surface bacteria (likely Gram-negative), the combined extract showed no inhibition at 25% and 50%. However, at 75%, inhibition increased (7.62 mm), suggesting potential synergy among plant compounds. Interestingly, at 100% concentration, inhibition dropped to 5.08 mm, which may indicate bacterial resistance or saturation effects where higher concentrations no longer enhance antibacterial activity (Wirahmi et al., 2021). This reduction indicates possible bacterial resistance or saturation of the bioactive compounds of the extract. As unwashed surfaces are usually inhabited by gram-negative bacteria, their outer membrane could have hindered the effectiveness of the extract. This corroborates (Igwe et al., 2023), in which



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Senna alata, *Psidium guajava*, and *Acalypha wilkesiana* extracts were found to have antibacterial activity, with efficacy varying by bacterial type and concentration, gram-negative bacteria proving more resistant. For soil bacteria tests, no inhibition was recorded at 25% and 50%, while 75% had variable results, ranging from moderate inhibition (7.62 mm) to none.

The reduced inhibition at 100% concentration (5.08 mm) aligns with previous studies (Wirahmi et al., 2021), which found that synergistic effects may be optimal at certain concentration ratios but diminish when bioactive compounds become too saturated or cytotoxic. This variation may indicate that the soil bacteria, comprising both gram-positive and gram-negative species, will have varying levels of resistance. The same results were obtained by (Wirahmi et al., 2021), who proved that the strongest antibacterial activity was shown by a 75% guava and 25% betel leaf mixture, suggesting that the best ratio of plant extracts could be necessary for successful antimicrobial activity. The general findings are that the combined extract had moderate antibacterial activity at 75% concentration against unwashed surface and soil bacteria but only weak inhibition at 100%, indicating a non-linear concentration-response. The lack of inhibition against yogurt bacteria (Gram-positive) suggests that the combined extract's active compounds were ineffective against Gram-positive cell structures. This is supported by (Thi et al., 2021), who



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noted that some plant compounds exhibit selective antibacterial activity depending on dose and bacterial type.

The inhibition variability calls for additional research on bacterial mechanisms of resistance, interactions among compounds, and enhanced extraction methods. This is reaffirmed by (Thi et al., 2021), whose study showed that *Piper betle* extract had dose-dependent biological activity, with some doses facilitating healing while the higher doses were cytotoxic. Future studies should aim at phytochemical profiling, novel extraction techniques, and increased bacterial testing to comprehensively evaluate the potential of the combined extract as a natural antibacterial agent.

The results of this research offer significant information regarding the antibacterial activity of *Psidium guajava* (Guava), *Piper betle* (Betel), and *Senna alata* (Acapulco) leaf extracts against different sources of bacteria. The results demonstrated that guava extract exhibited moderate antibacterial activity, particularly against Gram-positive yogurt bacteria and, to a lesser extent, soil bacteria. This suggests that bioactive compounds such as flavonoids and tannins contribute to bacterial inhibition, aligning with previous studies on plant-derived antimicrobials (Alam et al., 2023). Nonetheless, betel and acapulco extracts revealed no considerable antibacterial activity, which implies that these plant extracts might need dissimilar extraction solvents or better concentrations to give any detectable effects. The combined extract



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demonstrated moderate inhibition at 75%, suggesting potential synergy between the bioactive compounds. However, the reduction in activity at 100% concentration suggests a non-linear dose-response relationship, where higher concentrations may lead to compound saturation or bacterial adaptation (Wirahmi et al., 2021). These results add to the literature on plant-derived antimicrobial compounds by establishing that some plant extracts can possess selective antibacterial activity based on type of bacteria and concentration.

The findings are consistent with the theory that Gram-positive bacteria are generally more sensitive to plant-derived antimicrobials than Gram-negative bacteria, perhaps because of cell wall differences. In addition, the non-linear inhibition zone vs. concentration relationship indicates that higher extract concentration does not necessarily increase antibacterial efficacy, a factor to be considered in the development of natural antimicrobials. The research also points to the promise of guava leaf extract as a natural antibacterial agent, especially for use in food preservation and traditional medicine. But the poor effectiveness of betel and acapulco extracts implies that additional improvement in extraction methods and compound isolation is necessary.

Also, the resistance of unwashed surface bacteria to all extracts indicates that plant antimicrobials could be ineffective against some environmental strains of bacteria, further highlighting the importance of broader antibacterial screening.



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Conclusion

This study aimed to assess the antibacterial activity of guava, betel, and acapulco leaf extracts against yogurt, soil, and unwashed surface bacteria. The disk diffusion technique was used to measure the inhibition zones (mm) in varying concentrations (25%, 50%, 75%, and 100%) to see how effective each extract is. The findings confirmed that guava extract exhibited moderate antibacterial activity, particularly against Gram-positive yogurt bacteria, while betel and acapulco extracts did not display significant inhibition against any bacterial strains. This suggests that guava possesses bioactive compounds effective against Gram-positive bacteria, whereas betel and acapulco may require optimized extraction techniques or different bacterial targets. The combined extract exhibited moderate antibacterial activity at 75%, particularly against unwashed surface and soil bacteria, but its effectiveness declined at 100%, suggesting a saturation effect or bacterial resistance adaptation. This aligns with studies indicating that synergistic effects among plant bioactives are concentration-dependent (Wirahmi et al., 2021). These results corroborate the theory that plant extracts can exhibit selective antibacterial activity, where Gram-positive bacteria are more vulnerable compared to Gram-negative strains.

Yet, the research also underscores the shortcomings of plant antimicrobials, especially when bacterial resistance is an issue. The antibacterial activities exhibited by guava extract show its promise as a



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natural antimicrobial agent, but more work needs to be done to optimize extraction processes and enhance its antibacterial activity. The findings also highlight the importance of investigating other extraction methods and screening a wider variety of bacterial strains to ascertain the entire potential of these plant extracts.

Recommendations

From the findings, the following recommendations can be given for practical use and further research. A phytochemical analysis should be conducted using High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) to identify the specific bioactive compounds responsible for antibacterial activity in guava, betel, and acapulco extracts. This would inform on what compounds are involved in inhibition and whether or not their concentration could be maximized for better efficiency. Further research should explore ethanol and methanol-based extraction methods to determine whether alternative solvents can enhance the bioavailability and potency of active antibacterial compounds.

Given its demonstrated antibacterial activity against Gram-positive bacteria, guava extract should be explored for potential applications in food preservation as a natural antibacterial additive, herbal medicine as a topical or oral antibacterial treatment, and natural disinfectant as an alternative to chemical sanitizers. But betel and acapulco extracts might



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need to be altered or blended with other plant extracts in order to enhance their antibacterial activity. Future studies should evaluate the efficacy of these plant extracts against clinically relevant bacterial pathogens, to determine their potential use in alternative antimicrobial therapies for bacterial infections.

From a wider viewpoint, this research helps make a contribution to sustainable antimicrobial studies by pointing out the potential and the limitations of antibacterial agents from plants. Future research must explore the long-term stability and efficacy of such extracts in practical contexts, such as food safety, wound management, and infection control. Researchers must also consider the interaction of plant extracts with synthetic antibiotics to establish if they can be combined with antibiotics to fight bacterial resistance.

Finally, this research lays a basis for continued investigation into plant antimicrobials and suggests the necessity for ongoing investigation into natural substitutes for chemical antibiotics. Although guava extract was promising, further studies are needed to maximize extraction processes, evaluate safety, and discover the complete extent of its applications against bacteria.



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APPENDICES

Appendix A: Research Instrument



Republic of the Philippines
Department of Education
Region III – Central Luzon
Schools Division of City of Malolos
MARCELO H. DEL PILAR NATIONAL HIGH SCHOOL
Bagong Bayan, City of Malolos, Bulacan

February 17, 2025

MA. VICTORIA C. VIVO, EdD, CESE
Secondary School Principal IV
Marcelo H. del Pilar National High School
Bagong Bayan, City of Malolos, Bulacan

Madam:

Good day!

As part of our Research Project requirement, we are conducting a study titled **"Integrating Psidium guajava (Guava), Piper betle (Betle), and Senna alata (Akapulko) Leaf Extracts: A Comprehensive Evaluation of Their Synergistic Antibacterial and Wound-Healing Potentials."**

We respectfully seek permission to conduct our research outside the school premises. This study focuses solely on the antibacterial properties of plant extracts through laboratory experiments and **does not involve human respondents**. All procedures will strictly follow safety and ethical research guidelines.

We appreciate your consideration and look forward to your approval. Thank you.

Respectfully,

Noted:

DELA CRUZ, RHIAN DENISSE V.

JOHN MARIE E. MALCO, EdD
Research Adviser

SALAZAR, ASHLEY NICOLE V.

BENJAMIN F. MIJARES III
OIC- Subject Group Head-Research

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SENIOR HIGH SCHOOL DEPARTMENT**Appendix B: Raw Data Tables**

- Measured inhibition zones (mm) for all plant extracts against different bacterial strains.

Group	Bacteria Strains	Plant Extract (S)	Concentrations	Numbers of Trials
1.	Bacteria from Soil	P. guajava	25%,50%, 75%,100%	3
2.	Bacteria from Soil	P. betle	25%,50%, 75%,100%	3
3.	Bacteria from Soil	S. alata	25%,50%, 75%,100%	3
4.	Bacterial from Soil	Combination of Guava,Betel, Acapulco	25%,50%, 75%,100%	3
5.	Bacteria from Yogurt	P. guajava	25%,50%, 75%,100%	3



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6.	Bacteria from Yogurt	P. betle	25%,50%, 75%,100%	3
7.	Bacteria from Yogurt	S. alata	25%,50%, 75%,100%	3
8.	Bacteria from Yogurt	Combination of Guava,Betel, Acapulco	25%,50%, 75%,100%	3
9.	Bacteria from Surface	P. guajava	25%,50%, 75%,100%	3
10.	Bacteria from Surface	P. betle	25%,50%, 75%,100%	3



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Concentratio ns	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standar d Deviatio n	Interpret ation
25%	0mm	10.16	0mm	3.38	5.86	No to minimal inhibitio n
50%	0mm	7.62m m	10.16m m	5.92	5.28	Mild inhibitio n
75%	0mm	10.16m m	10.16m m	6.77	5.86	Moderate inhibitio n
100%	0mm	5.08m m	0mm	1.69	2.93	No to minimal inhibitio n

GUAVA AGAINST UNWASHED SURFACE BACTERIA

Concentratio ns	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standar d Deviatio n	Interpret ation
25%	0mm	0mm	0mm	0	0	No inhibitio n zone
50%	0mm	0mm	0mm	0	0	No inhibitio n zone



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75%	0mm	0mm	0mm	0	0	No inhibition zone
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ACAPULCO AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
ACAPULCO AGAINST SOIL BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone



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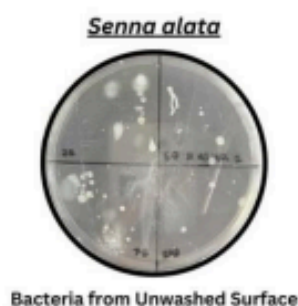
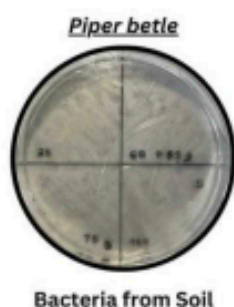
100%	0mm	0mm	0mm	0	0	No inhibition zone
ACAPULCO AGAINST UNWASHED SURFACE BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	0mm	0mm	0	0	No inhibition zone
100%	0mm	0mm	0mm	0	0	No inhibition zone
COMBINED EXTRACTS AGAINST SOIL BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	0mm	7.62mm	7.62mm	5.08	0	No to moderate inhibition
100%	5.08mm	5.08mm	5.08mm	5.08	0	Mild inhibition
COMBINED EXTRACTS AGAINST YOGURT BACTERIA						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation

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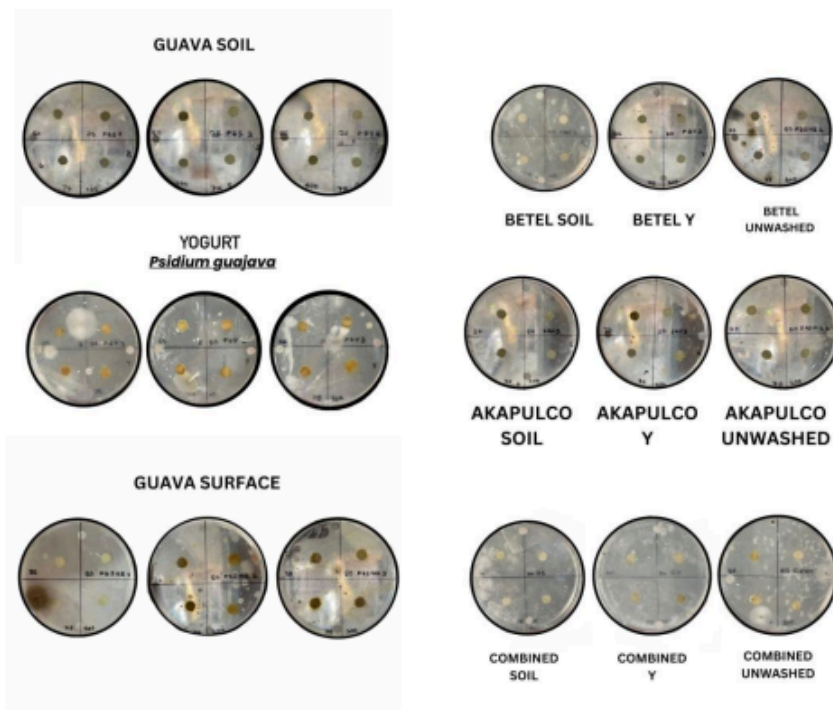
25%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
50%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
75%	0mm	0mm	0mm	0mm	0mm	No inhibition zone
100%	5.08mm	5.08mm	5.08mm	5.08mm	0mm	Moderate inhibition
COMBINED EXTRACTS AGAINST UNWASHED SURFACE						
Concentrations	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean	Standard Deviation	Interpretation
25%	0mm	0mm	0mm	0	0	No inhibition zone
50%	0mm	0mm	0mm	0	0	No inhibition zone
75%	7.62mm	7.62mm	7.62mm	7.62	0	Moderate inhibition
100%	5.08mm	5.08mm	5.08mm	5.08	0	Moderate inhibition

Appendix C: Photographic Documentation

- Images of bacterial growth



- Inhibition zones in Agar plates



Appendix

- Biosafety Guidelines Followed (the use of environmental bacteria instead of pathogenic strains).

Sterilization and Disposal Methods:



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- Used agar plates were soaked in bleach or 70% alcohol before disposal.
- Glassware and materials were disinfected and sterilized after use.

Appendix E: Statistical Analysis

- Mean and standard deviation calculations for inhibition zones.