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Antibacterial Efficacy of Bagras (Eucalyptus Deglupta Blume) and Makopa (Syzygium Samarangense [Blume] Merr. & L.M. Perry) Leaf Ethanolic Extract Against Pseudomonas Aeruginosa

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ABSTRACT

The fluctuating spread of antibiotic-resistant Pseudomonas aeruginosa has rendered the development of alternative treatments against the global threat a necessity. Accordingly, this study aimed to assess the antibacterial efficacy of ethanolic extracts of bagras (Eucalyptus deglupta Blume) and makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry) leaves against P. aeruginosa. This study utilized a true experimental posttest-only design using the surface application method to evaluate the antibacterial efficacy of the ethanolic leaf extracts against the bacteria. After 24 hours of incubation at 37°C, the zone of inhibition (ZOI) was measured and analyzed to determine antibacterial activity. Findings show that bagras extract exhibited strong antibacterial activity, with 21–31mm ZOI. In contrast, makopa extract was ineffective, with no ZOI observed; however, its combination with bagras showed enhanced efficacy, ranging from 25–34mm ZOI, suggesting a synergistic effect. Compared to Tobramycin, a commercial antibiotic, both bagras alone and in combination with makopa demonstrated significant differences in antibacterial activity, highlighting their potential as natural antibacterial agents. These findings serve as valuable reference for the Department of Health and the Department of Science and Technology in constructing strategies to combat the spread of *P. aeruginosa*. Likewise, it also provides insights into the antibacterial potential of bagras and makopa, providing a framework for pharmacologists and medical professionals in developing potent herbal treatments against multidrug-resistant bacteria. Additionally, it encourages further research into the phytochemical properties and synergistic effects of these plants, contributing to advancements in medicinal chemistry, pharmacology, and global health.

Keywords: Antibiotic resistance, Pseudomonas aeruginosa, Bagras, Makopa, Antibacterial, Quantitative Research

INTRODUCTION

Cleanliness is essential for maintaining good health by minimizing exposure to harmful germs and reducing the risk of diseases. However, bacteria are everywhere, occurring in both beneficial and harmful forms, in different parts of the body, in the soil, and in moist environments. For instance, individuals with skin injuries like cuts and burns may come into contact with contaminated water or soil through activities like swimming and gardening. This exposure can introduce bacteria that may lead to respiratory, urinary tract, skin, and bloodstream infections, which can be challenging to treat due to antibiotic resistance. Additionally, the natural antibacterial properties of plants remain underutilized due to limited research into their potential, and questions about their efficacy and safety compared to manufactured antibiotics.

Globally, gram-negative bacterium like *Pseudomonas aeruginosa* poses a major threat in healthcare settings and is a common cause of infections. In the study of Thomsen et al. (2023), *P. aeruginosa* was found in approximately 24% of ICU-acquired infections worldwide, specifically reaching 48.7% in Brazil. In South

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Africa, patients with cystic fibrosis (CF) are highly susceptible to infections by multidrug-resistant isolates of *P.* aeruginosa, with 60-80% of CF adults showing chronic infection by this pathogen (Parkins et al., 2018; Hamiwe et al., 2024). As well as patients with community-acquired pneumonia (CAP) in Croatia, the rate of P. aeruginosa CAP in patients with prior infection caused by P. aeruginosa and at least one chronic lung disease was 67%. As mentioned by Garousi et al. (2023), in African and Western countries, the prevalence rates of P. aeruginosa in diabetic foot ulcer infections were reported at 16.3% and 11.1% respectively. This indicates how widespread this pathogen is globally and how it continues to be a healthcare burden.

Additionally, the prevalence of antibiotic-resistant P. aeruginosa increases morbidity, limiting treatment options. The Pan American Health Organization/World Health Organization (PAHO/WHO) received a report concerning surgical site infections caused by antibiotic-resistant P. aeruginosa after invasive procedures were performed in Tijuana, Mexico (WHO, 2019). In a review, P. aeruginosa isolates from the Middle East and North Africa region, especially Saudi Arabia, Egypt, Libya, Syria, and Lebanon showed high-level resistance to different antibiotics, particularly in critical care units (Momenah et al., 2023). In Spain, approximately 30% of all *P. aeruginosa* strains from infections acquired in Spanish ICUs are resistant to carbapenems, ceftazidime, and quinolones. The increasing spread of highly resistant bacteria underscores the urgent need for improved treatments and more potent therapeutic solutions.

In Southeast Asia, healthcare-acquired infections (HAI) caused by *P. aeruginosa* are significant health concerns affecting vulnerable populations. Notably, HAI caused by *P. aeruginosa* in Southeast Asia is high, at approximately 22%, which is at the higher end of the worldwide scale, with Indonesia having the highest prevalence rate of HAI at 30.4% in the region. This HAI can be attributed to *P. aeruginosa*, which made up 13.8% of hospital-acquired pneumonia in Thailand.

This rise of P. aeruginosa infections has also exhibited antibacterial and antimicrobial resistance, making various treatments in the region ineffective. In Indonesia alone, a total of 1554 P. aeruginosa isolates present resistance to multiple antibiotics, including colistin, an antibiotic used for HAI, was found to be 100% ineffective against the bacteria. Furthermore, P. aeruginosa isolates exhibited broad-spectrum resistance (87.8% multidrug resistance) in a public acute care hospital in Singapore, remaining only susceptible to polymyxin B (95.0%) and amikacin (55.0%). This information urges a need to address the complications and issues caused by *P.* aeruginosa.

Given these concerns, the global burden of P. aeruginosa infections poses a rising challenge because of antibiotic resistance driving the development of a wide range of strategies to address it. Fiel and Roesch (2022) mentioned in their study that aminoglycosides like tobramycin exhibit effectiveness against the Gram-negative bacteria, including P. aeruginosa. A study by Reynolds and Kollef (2021) also demonstrated that patients who received 300mg of tobramycin twice daily for 28 days eradicated P. aeruginosa in more than 70% of patients while improving lung function. To add, aminoglycosides, including amikacin, gentamicin, and tobramycin are usually used to treat urinary tract infections caused by P. Aeruginosa.

Additionally, the presence of multiple beneficial phytochemicals in medicinal herbs like bagras aid in the successful process of inhibiting various bacteria. From the study of Kareem et al. (2020), three varying concentrations of bagras ethanolic extracts against another gram-negative bacterium, *E. coli*, resulted in 18 mm, 20.5 mm, and 22.5 mm of zone of inhibitions (ZOIs). This finding aligns with the study by Abiodun et al. (2024), which highlighted the strong antibacterial effects of eucalyptus essential oil on several respiratory pathogens, including *E. cloacae* with a mean ZOI of 23.0 mm, *K. pneumoniae* with 22.7 mm, and *S. aureus* with 16.0 mm, at a concentration of 100mg/mL. On the other hand, makopa showed weak to no signs of the zone of inhibitions against bacteria similar to the mentioned studies above. The makopa leaves ethanol extract presented weak inhibitory activity against Gram-negative bacteria such as *E. coli* showing 7 mm ± 0.99 mm and 9 mm ± 0.89 mm diameters of ZOIs respectively (Khandaker et al., 2015). This indicates that the bagras leaves have a greater potential of being an antibacterial agent against pathogens compared to makopa leaves.

The Philippines has also been subjected to the spread of *P. aeruginosa*, contributing to the growing cases of diseases related to the said bacteria. In post-percutaneous nephrolithotripsy, it is highlighted that *P. aeruginosa* is the most common bacteria in kidney stones in the Philippines, noting its high resistance to standard

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antibiotics. DomA rise of carbapenem-resistant *P. aeruginosa* in three tertiary hospitals in Metro Manila. To be precise, Chilam et al. (2021) explained that this continual spread of multidrug-resistant P. aeruginosa, complicates effective infection control and treatment in the country. To address these issues, traditional medical treatments for P. aeruginosa in the country include piperacillin-tazobactam. However, with the influx of varying, multidrug-resistant strains of P. aeruginosa in 2023, the efficacy of these treatments has been significantly compromised (Antimicrobial Resistance Surveillance Reference Laboratory [AMRSRL], 2023).

Looking at the issue in a local lens, the spread of P. aeruginosa is not a minor matter, as the bacterium was detected in various sources. In Davao City, carbapenemase-producing P. aeruginosa has been recorded in the city's coastal wetlands and in a tertiary-level private hospital, with samples coming from patients' endotracheal aspirates and wounds, signifying a concerning surge of carbapenem-resistant P. aeruginosa within the city, and the potential for its spread to neighboring areas. With this rising threat of P. aeruginosa, researchers have explored the antibacterial activity of widely available plants in Mindanao as means to combat the bacterium. As a case in point, extracts from roots of Tabernaemontana pandacaqui Poir (pandakaki-puti) as well as essential oils from P. guajava (bayabas) leaves and L. domesticum (lanzones) pericarp displayed ZOIs of up to 14.8mm, 12.7mm, and 12.9mm against P. aeruginosa, respectively (Sicalan et al., 2023). While these findings suggest that P. aeruginosa can be addressed using readily available natural resources in the locality, their efficacy falls short in the face of both the bacterium's proliferation and multidrug resistance.

Accordingly, the rising concern of antibiotic resistance of such bacteria has spurred an exploration into the antibacterial potential of the bagras as a source of novel therapeutic agents. Several studies highlight the efficacy of bagras extracts against various bacterial pathogens. For instance, Hapid et al. (2024) demonstrated that ethanolic leaf extracts of bagras effectively inhibited the growth of *S. aureus* with inhibition zones of 15.67 mm and 19.32 mm at 25% and 100% concentrations, respectively. The practical applicability of these findings is further supported by Winarti et al. (2024), who developed a mouthwash nanoemulsion combining eucalyptol from bagras with peppermint oil and found significant antibacterial activity against both *E. coli* and *S. aureus*. These results suggest bagras has significant antibacterial properties worth further study.

On the other hand, makopa has also been gaining significant attention for its potential health benefits. The presence of various bioactive compounds in makopa leaves including flavonoids, phenolic compounds, alkaloids, and saponins supports its medical and therapeutic potential. These bioactive compounds are important due to their diverse biological activities, including antibacterial, antioxidant, and anti-inflammatory (Tarigan et al., 2022; Jayakumari et al., 2023). However, the ethanolic extract of makopa leaves has demonstrated little to no susceptibility against few bacteria. As such, from the study of Khandaker et al. (2015), the makopa leaf ethanol extract showed weak inhibitory activity against E. coli showing 7 mm ± 0.99 mm and 9 mm ± 0.89 mm diameters of ZOIs respectively. The study of Sulthana et al. (2023) showed similar outcomes as well, P. aeruginosa showed resistance against the makopa ethanolic extract with 8 mm and 9 mm diameters of ZOIs.

To sum up, a highly drug-resistant Gram-negative bacterium like P. aeruginosa, notorious for causing infections in the lungs, urinary tract, and other body parts, should urgently be addressed as this bacterium is particularly harmful to vulnerable populations, including immunocompromised individuals and the elderly. The spread of this bacterium is a global health issue encapsulated in the third Sustainable Development Goal, focusing on good health and well-being, rendering a heightened need for new treatments against it. Due to its complexity, however, there is a shortage of further investigation regarding possible alternatives, specifically the plants bagras and makopa, for controlling the spread of P. aeruginosa compared to the currently available treatments, such as aminoglycosides and beta-lactam antibiotics. Hence, this study aimed to delve deeper into the antibacterial efficacy of the ethanolic extract of bagras (Eucalyptus deglupta Blume) and makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry) leaves against P. aeruginosa.

Theoretical Framework

This research is anchored on Robert Koch and Louis Pasteur's Germ Theory of Disease (1861). Robert Koch's postulates are essential criteria in proving that specific microorganisms cause specific diseases. This indicates that specific microorganisms become invariably present in diseased individuals and absent in healthy ones,

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capable of being isolated and reproduced experimentally (Segre, 2013). This concept was seminal in underpinning Louis Pasteur's germ theory of disease, which has since been elaborated to explain how *P. aeruginosa*— a bacterial pathogen utilized in this study, causing pneumonia, UTI, and various other infections— brings about disease (Liddell & Hartsock, 2023).

This study is also anchored on the theory of non-random selection of medicinal plants, proposed by Daniel Moerman in 1979. The theory propounds that the selection and usage of plants for traditional medicinal methods are hinged on their curative properties and effectiveness. Those characteristics are said to be empirical, which means that over time, taxonomic groups that share similar medicinal qualities are bound to be prominently utilized. In Bagras (Eucalyptus deglupta Blume) and Makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry) leaves, high amounts of monoterpene hydrocarbons are observed, which are known to synthesize antibacterial activity, thus supporting the use of the herbs against P. aeruginosa in this study (Insuan et al., 2021).

In summary, this study is anchored on two theories that accurately substantiate the use of bagras and makopa leaves as well as the utilization of P. aeruginosa. By isolating the bacterium and testing its response to ethanol extracts, the researchers aim to demonstrate a direct antibacterial effect, thus supporting the germ theory of disease. Assessing the antibacterial properties of bagras and makopa against P. aeruginosa gives grounds for utilizing the said herb in the study, supporting Moerman's theory of non-random selection of medicinal plants.

Statement of the Problem

The main purpose of the study was to determine the efficacy of different ethanolic extract concentrations of bagras (Eucalyptus deglupta Blume) and makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry) against P. aeruginosa. Specifically, it aimed to assess whether there was a significant difference in inhibiting bacterial growth when using different concentrations of the extract compared to the positive control group.

1. What is the level of the antibacterial efficacy of the different concentrations of bagras leaf ethanolic extract against Pseudomonas aeruginosa in terms of:

   1.1 40 mg/mL concentration of bagras leaf ethanolic extract;

   1.2 60 mg/mL concentration of bagras leaf ethanolic extract; and

   1.3 80 mg/mL concentration bagras leaf ethanolic extract?

2. What is the level of the antibacterial efficacy of the different concentrations of makopa leaf ethanolic extract against *Pseudomonas aeruginosa* in terms of:

   2.1 40 mg/mL concentration of makopa leaf ethanolic extract;

   2.2 60 mg/mL concentration of makopa leaf ethanolic extract; and

   2.3 80 mg/mL concentration of makopa leaf ethanolic extract?

3. What is the level of the antibacterial efficacy of the different concentrations of bagras and makopa leaf ethanolic extract against *Pseudomonas aeruginosa* in terms of:

   3.1 40 mg/mL concentration of the combination of bagras and makopa leaf ethanolic extract;

   3.2 60 mg/mL concentration of the combination of bagras and makopa leaf ethanolic extract; and

   3.3 80 mg/mL concentration of the combination of bagras and makopa leaf ethanolic extract?

4. What is the level of the antibacterial efficacy of the commercial antibiotic, Tobramycin, against Pseudomonas aeruginosa?

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5. Is there a significant difference in inhibiting the growth of P. aeruginosa using the different concentrations of the ethanolic extract of bagras (Eucalyptus deglupta Blume) and makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry) leaves compared to the control treatment Tobramycin?

Hypothesis

To objectively answer the problem listed in the preceding section, the given null hypotheses was formulated:

H_o: There is no significant difference in the effectiveness between bagras (*Eucalyptus deglupta* Blume) and makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaf ethanolic extract on the inhibition of *Pseudomonas aeruginosa.*

H_{o1}: There is no significant difference in the effectiveness between bagras (*Eucalyptus deglupta* Blume) and makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaf ethanolic extracts and control treatment on the inhibition of *Pseudomonas aeruginosa.*

Significance of the Study

This study explored the efficacy of bagras (*Eucalyptus deglupta* Blume) and makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaves ethanolic extract inhibiting the growth of *P. aeruginosa.* Therefore, the researchers conducted this study, which could be beneficial to the following:

Department of Health Officials. This study can give new insights to the Department of Health about the antibacterial efficacy of bagras and makopa leaves as a potential inhibitor against different infections. The Department of Health can use the data and results gathered to investigate bagras and makopa leaves and their efficacy in inhibiting the spread of *P. aeruginosa* further, which could improve public health initiatives of healthcare professionals to respiratory diseases.

Department of Science and Technology Officials. The findings of this study can give insights to the Department of Science and Technology by exploring the antibacterial properties of the bagras and makopa leaves as inhibitors of *P. aeruginosa* infection that could help in advancing research involving bacterias. Moreover, this could further support research and innovations in the field of bacteriology.

Pharmacologists. The results gathered from this study can provide insights into the pharmaceutical industry by exploring the antibacterial properties of the bagras and makopa leaves as inhibitors of *P. aeruginosa* infection that could also serve as a basis for developing innovative plant-based medicines alternatives. Furthermore, this could be helpful to the pharmaceutical industry to further improve alternative treatments against drug-resistant pathogens.

Medical Professionals. The findings of this study can assist medical professionals to contribute to the development of natural alternative treatment for infections caused by *P. aeruginosa* in partnership with the pharmaceutical industry. Additionally, this study could provide knowledge about bacterial resistance resulting in improved treatment formulations and encouraging further studies into natural plant-based treatments.

Future Researchers. This study can provide comprehensive knowledge to future researchers that could aid new possibilities to the antibacterial properties of bagras and makopa leaves and their efficacy in inhibiting the growth of highly drug-resistant pathogens like *P. aeruginosa*. The findings of this study can serve as a helpful reference for future developments.

Scope and Limitations

This study focused on investigating the antibacterial properties of ethanolic extracts from bagras (*Eucalyptus deglupta Blume*) and makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaves against *P. aeruginosa.* The fresh bagras and makopa leaves were sourced locally within Davao del Sur. The research was conducted during the first and second semesters of the academic year 2024-2025 in laboratories within Davao

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del Sur. The antibacterial activity was determined by culturing *P. aeruginosa* on Mueller-Hinton agar plates and incubating at 37 °C. After 24 hours, the zone of inhibition was measured in millimeters.

However, this study had certain limitations. Primarily, this study was limited to the ethanolic extracts of bagras and makopa leaves and their antibacterial activity solely against *P. aeruginosa.* Other extraction methods and solvents were not explored. Furthermore, the focus of the study was on *P. aeruginosa*, meaning that other bacterial species were excluded from the analysis. In terms of methodology, the use of laboratory equipment and materials was also a limitation, as the study specifically focused on using Mueller-Hinton agar plates. Moreover, the geographic scope was limited to Davao del Sur for sourcing plant materials, potentially affecting the generalizability of the findings to bagras and makopa leaves from other regions.

Definition of Terms

To have a clearer understanding of this study, the following terminologies were conceptually and operationally defined:

Antibacterial. It refers to the ability of a substance to destroy or suppress the growth of bacteria and their ability to reproduce. In this study, it is the ability of the ethanolic extract of bagras and makopa leaves to inhibit the growth of *P. aeruginosa* that was assessed according to the zone of inhibitions.

Bagras (*Eucalyptus deglupta* Blume). It is famous for its multicolored trunk, also known as Mindanao gum. It thrives in the biodiverse rainforests of the Philippines, Indonesia, and Papua New Guinea which can grow to more than 1,800 meters and is the only eucalyptus species in the Northern Hemisphere. In this study, the antibacterial efficacy of its leaf ethanolic extract against *P. aeruginosa* was evaluated using the disk diffusion method.

Ethanolic extract. It is a preparation obtained by using ethanol as a solvent to dissolve and separate soluble compounds from a source material, typically plant matter. In this study, this refers to the type of solution which the bagras and makopa leaves were made from to determine its antibacterial efficacy against *P. aeruginosa*.

Makopa (Syzygium samarangense [Blume] Merr. & L.M. Perry). It is also known as the wax apple, a tropical tree native to Southeast Asia including the Philippines, it bears a bell-shaped fruit in various colors with crisp, apple-like flesh. In this study, this refers to the antibacterial properties of the ethanolic extract of its leaves will be evaluated using the disk diffusion method.

Pseudomonas aeruginosa. It is a Gram-negative, rod-shaped, opportunistic bacteria notable for its antibiotic resistance and role as a frequent cause of hospital-acquired infections. In this study, this is the pathogenic bacteria that was utilized in the experiment and examined using the dosc diffusion method.

Surface Application Method. It is when the surface application method in antibacterial testing involves directly applying a measured amount of a substance onto the surface of an inoculated agar plate, allowing it to diffuse and inhibit bacterial growth, which is then assessed by measuring zones of inhibition. In this study, the extract of Bagras, Makopa, and their combination was directly applied to the inoculated agar plate in a 30 µL. This was done to assess their potential antibacterial activity.

Zone of Inhibition. It is an area of media where the microorganisms are unable to reproduce, due to the presence of a drug that prevents their ability to grow. In this study, this refers to an area on the MH agar plates where the *P. aeruginosa* cannot grow, because of the presence of antibacterial agents such as bagras and makopa leaf ethanolic extract

METHODS

This chapter provides the subject of the study, sampling methodologies, sources of data, procedures for data collection, measurement techniques, methods of analysis and interpretation, and ethical considerations.

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Research Design

In this study, a true experimental quantitative research design was utilized, specifically posttest-only. A true experimental quantitative design was essential as this study's main objective was to assess the antibacterial efficacy of bagras and makopa leaf ethanolic extract by quantifying the means of the zone of inhibition of each extract against *Pseudomonas aeruginosa*. By manipulating the three (3) independent variables and observing the effects on the dependent variable, the researchers were able to draw conclusions about how changes in one factor influence another (Bhat, 2018).

Moreover, this study specifically utilized a posttest-only research design. The researchers applied the bagras and makopa leaf ethanolic extracts to the *P. aeruginosa* strains and then measured the independent variables' effect on the dependent variable without conducting any pretests. In this particular research design, subjects received specific interventions or treatments, and only after the intervention were their results assessed (Birt et al., 2022). This design allowed researchers to evaluate changes in bacterial growth following exposure to the plant extract without requiring a baseline measurement.

Subject of the Study

This study used Gram-negative bacteria, known as *P. aeruginosa*, as the subject. For this study, a total of 30 *P. aeruginosa* cultures were required, with three experimental groups and one positive control group. Each experimental group consisted of nine bacteria cultures, with three cultures for each concentration of the leaf ethanolic extract of bagras, makopa, and a combination of both. Meanwhile, the positive control group consisted of three bacteria cultures. The experiment was replicated thrice to ensure the validity and significance of the result. Furthermore, the study was carried out in a microbiology laboratory in Digos City, Davao del Sur.

Sampling Technique

In this study, the researchers utilized the complete random sampling technique to select subjects in the experiment. Complete random sampling involves selecting bacterial samples in a way that ensures complete randomness to avoid any systematic bias in the sampling process (Ma & Tu, 2022). This method enabled each strain to have an equal chance of being chosen, thus guaranteeing an unbiased and accurate representation of the subset of bacteria used for study analysis.

According to Horton (2024), this technique allowed the researchers to statistically measure a subset of subjects selected from a larger population to approximate a response from the entire group. This approach contributed to the preservation of strong internal validity by lowering the risk of research biases through randomization. Hence, the application of this method greatly improved the validity and reliability of the study's outcome, ensuring that it chose an accurate representative subset of *P. aeruginosa*.

Measures

This quantitative study adopted the disk diffusion method introduced by Gomes et al. (2021). In this method, the plates undergo a 24-hour incubation period at 37 °C, and the diameter of the zone of inhibition (ZOI) surrounding each extract was measured with a ruler to assess the antibacterial activity of the plates. The evaluation was conducted by an experienced medical laboratory technologist with over two years of expertise in bacterial infection studies, who specifically examined the efficacy of the ethanolic extract of bagras and makopa leaves in inhibiting *P. aeruginosa* growth. This research established the antibacterial activity of the leaf extract by monitoring the duration of bacterial growth inhibition. Additionally, the ZOI was taken to determine the degree of the antibacterial activity of the extract through the criteria of Hudzicki (2016), wherein an inhibition zone size less than 14 mm indicates resistance, while those 15 to 16 mm reflects moderate activity, and a zone of 17 mm above signifies high sensitivity to the extract.

The primary objective of this evaluation was to assess the efficacy of bagras and makopa leaf extracts in inhibiting the growth of *P. aeruginosa*, focusing on their antibacterial properties and inhibition zones. The

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technologist's assessment was focused on evaluating parameters based on a standardized scale to determine the effectiveness of the leaf extracts in inhibiting the growth of *P. aeruginosa*. Post-assessment, the compiled evaluations were organized using an interpretation table and subjected to systematic analysis. This analytical approach aimed to evaluate the extracts' potential influence on bacterial growth inhibition, highlighting its relevance for future applications against *P. aeruginosa*.

Table 1. Pseudomonas aeruginosa's Growth Inhibition Interpretation

Mean Score Interval	Descriptive Equivalent	Interpretation
≥17 mm	Susceptible	The antibacterial activity showed an excellent image of the zone of inhibition.
15.00 mm - 16.00 mm	Moderate	The antibacterial activity showed a fair image of the zone of inhibition.
≤14 mm	Resistant	The antibacterial activity showed poor image of the zone of inhibition.

Data Gathering Procedure

In the process of gathering data for this study, the researchers followed specific procedures to acquire information.

Extraction of Bagras (*Eucalyptus deglupta* Blume)

The following procedures were adapted from the study of Hapid et al. (2024):

1. The researchers obtained bagras tree leaves from the City Environment and Natural Resources Office (CENRO) Digos City and gathered and washed sufficient quantities of leaves, ensuring they were free from dirt and contaminants.
2. The leaves were dried using an air fryer and oven while maintaining a controlled temperature and crushed using a blender to create a coarse powder.
3. The researchers used an electronic weighing scale for each leaf powder to ensure equal measurements.
4. After doing so, the leaf powders for the 40 mg/mL, 60 mg/mL, and 80 mg/mL concentrations were placed in separate glass jars, and then each was mixed with 50 mL of 95% ethanol.
5. The sealed jar was left to macerate at room temperature for approximately 120 hours with gentle shaking from time to time, after which the mixture was filtered with filter paper to separate the liquid extract from the solid residue.
6. To evaporate the ethanol, some of the extracts were soaked in a water bath while some were soaked in a large beaker placed on a hot plate with a magnetic stirrer for an hour at 40°C.
7. The ethanol extract was stored in a glass jar at 4°C to protect it from light and degradation.
8. All materials utilized in the extraction process were sterilized to prevent bacterial contamination.

Extraction of Makopa (Syzygium samarangense [Blume] Merr. & L. M. Perry)

The following procedures have been adapted from the research of Idris et al. (2023).

1. The researchers collected makopa tree leaves directly from a nearby backyard. The branches were separated since only the leaves were utilized in the study.
2. The leaves were thoroughly washed using clean running water to remove any dirt or impurities, then it was dried in an oven and air fryer at a controlled temperature, and crushed into a coarse powder using a blender.
3. The researchers used an electronic weighing scale for each leaf powder to ensure equal measurements.

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4. Each weighed leaf powders for the 40 mg/mL, 60 mg/mL, and 80 mg/mL were placed in separate glass jars, and was mixed with 50 mL of 95% ethanol.
5. The sealed jar was left to macerate at room temperature for approximately 120 hours with gentle shaking from time to time, after which the mixture was filtered with filter paper to separate the liquid extract from the solid residue.
6. To evaporate the ethanol, some of the extracts were soaked in a water bath while some were soaked in a large beaker placed on a hot plate with a magnetic stirrer for an hour at 40°C.
7. The ethanol extract was stored in a sealed glass jar at 4°C until further analysis.
8. All materials utilized in the extraction process were sterilized to prevent bacterial contamination.

Extraction of Bagras (Eucalyptus deglupta Blume) **and Makopa** (Syzygium samarangense [Blume] Merr. & L.M. Perry)

The following procedures were adapted from the study of Hapid et al. (2024) and Idris et al. (2023).

1. The researchers obtained the bagras tree leaves from the City Environment and Natural Resources Office (CENRO) Digos City, while the makopa tree leaves were acquired from a nearby backyard.
2. The leaves were separated from the branches, then the researchers washed them thoroughly to ensure they are free from dirt and contaminants.
3. The leaves were then dried using an air fryer and an oven at a controlled temperature, and was crushed into a coarse powder using a blender.
4. The researchers used an electronic weighing scale for each leaf powder to ensure equal measurements.
5. Each of the leaf powders for the 40 mg/mL, 60 mg/mL, and 80 mg/mL were then placed separately into sanitary glass jars and were mixed with 50 mL of 95% ethanol.
6. The sealed jar was left to macerate at room temperature for approximately 120 hours with gentle shaking from time to time, after which the mixture was filtered with a filter paper to separate the liquid extract from the solid residue.
7. To evaporate the ethanol, some of the extracts were soaked in a water bath while some were soaked in a large beaker placed on a hot plate with a magnetic stirrer for an hour at 40°C.
8. The ethanol extract was stored in a sealed glass jar at 4°C until further analysis.
9. All materials utilized in the extraction process were sterilized to prevent bacterial contamination.

Preparation of Pseudomonas aeruginosa and Culture Media

The following procedures were adapted from the study of Kareem et al. (2020)

1. Before actuating the inoculation for the bacterial cultures, the researchers wore complete Personal protective equipment (PPE) which includes a laboratory gown, gloves, and face mask to maintain and ensure proper handling and safety.
2. Then, the *P. aeruginosa* bacteria cultures were obtained from a laboratory in a local hospital, which they had acquired from the Department of Health (DOH).
3. 30 MH agar plates were prepared and used as a medium for storage and culture for the bacteria set-up.
4. A culture tube containing a normal saline solution was prepared and inoculated with cultures of *P. aeruginosa*.
5. Each MH agar plate was evenly streaked on its surface using a sterile swab that had been dipped into the inoculum.

Evaluation of Antibacterial Activity by Surface Application Method

The following procedures were adapted from the studies of Lyons et al. (2020)

1. The Mueller-Hinton agar plates that had been streaked with *P. aeruginosa* were then inoculated with the prepared extracts.

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2. Each trial extract was precisely measured at 30 µL using a sterile electronic micropipette, which was then used to apply the extract to the respective MH agar plates.
3. For the antibiotic tobramycin, exactly 40 µL (equivalent to one drop) was applied to the MH agar plates.
4. The inoculated Mueller-Hinton agar plates were left at 37°C for 24 hours to allow the extracts to diffuse and interact with the bacteria. After this incubation period, the plates were assessed for antibacterial activity by measuring the zone of inhibition around each extract using a ruler.

Analysis and Interpretation

To analyze the data of this experimental study, the researchers will measure the means, as well as utilize both the Analysis of Variance (ANOVA) and Tukey Honestly Significant Difference (HSD) test method to further investigate the distinction between three independent variables based on one dependent variable.

1. The mean serves as an indicator of the average value calculated from a set of given results from each concentration, measured in millimeters. Statistical analyses often rely on comparing means to determine if there are significant differences between groups (Saha, 2024).
2. Analysis of Variance (ANOVA) is a statistical method used to compare the means of three or more groups to determine if there are any statistically significant differences among them (Hassan, 2024). A one-way ANOVA was utilized to determine the significant difference between the antibacterial properties of the ethanolic extract of bagras, makopa, and a combination of both plant extracts against P. aeruginosa.
3. Tukey Honestly Significant Difference (HSD) Test is a statistical tool that was used to assess if the relationship between sets of data is statistically significant and to identify specific differences between group means. This means that it evaluates whether there's a strong chance that the numerical change in one variable is causally related to an observed change in another variable (Beck, 2022). After the demonstration of the ANOVA, the HSD Test was then established to statistically verify which among the concentrations is most and least effective against P. aeruginosa.

Ethical Considerations

This study upheld firm ethical standards to ensure responsible and conscientious research practices. Such considerations were implemented to uphold the study's integrity, ensure the proper management of biological materials, and comply with scientific, environmental, and institutional standards.

Biosafety and risk management. is vital in ensuring the safety of the researchers, as well as other individuals involved in this study. In particular, strict adherence to laboratory regulations in both institutional and hospital facilities helps minimize exposure to harmful agents. As stressed by the National Institutes of Health (2021), this also entails appropriate handling and storage of extracts, substances, laboratory apparatus, as well as the disposal of utilized agar plates and expendable personal protective equipment, thereby maintaining a safe and controlled research environment.

Environmental responsibility and sustainability. are fundamental considerations in this study, ensuring that the research practices do not harm any ecosystems associated with the study. In particular, the researchers ensured that the extraction of bagras and makopa leaves and the bacterial culturing of *P. aeruginosa* do not negatively affect or contribute to the degradation of the environment (Dapar et al., 2020).

Integrity and transparency. are significant qualities of a study that ensures all research processes are conducted at utmost honesty and without alteration or misrepresentation. Throughout the stages of this research endeavor, from assessing the antibacterial efficacy of bagras and makopa leaf ethanolic extracts against *P. aeruginosa* to evaluating the findings of said experiments, the researchers put morality and respect as their footing in taking part in the data collection, analysis, and reporting (Zhaksylyk et al., 2023)

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RESULTS AND DISCUSSION

This chapter deals with the presentation, analysis, and interpretation of data. The first part describes the levels of antibacterial activity of the different concentrations of bagras (*Eucalyptus deglupta* Blume) leaf ethanolic extract, makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaf ethanolic extract, combined bagras and makopa leaf ethanolic extract, and the control group, tobramycin. The second part describes the significance of the differences in the effectiveness among the different concentrations in inhibiting the growth of *P. aeruginosa,* including comparisons with the control treatment and the Post Hoc Comparison using the Tukey HSD test and a one-way ANOVA.

Antibacterial Efficacy of the Different Concentrations of Bagras Leaf Ethanolic Extract on the Pseudomonas aeruginosa

The study determined the effectiveness of the bagras leaf ethanolic extract on the growth inhibition of *P. aeruginosa* with three different treatments: Treatment 1 - 40 mg/mL concentration of bagras leaf ethanolic extract; Treatment 2 - 60 mg/mL concentration of bagras leaf ethanolic extract; and Treatment 3 - 80 mg/mL concentration of bagras leaf ethanolic extract. The researchers determined its antibacterial efficacy by measuring the zone of inhibition per treatment in each replication. Hence, the researchers obtained the following results.

Table 1. Antibacterial Efficacy of the Different Concentrations of Bagras Leaf Ethanolic Extract on the Pseudomonas aeruginosa

Treatments	Zone of Inhibition (in mm)			Mean	SD	Description
	R1	R2	R3			
T1	30	29	21	26.67	4.93	Susceptible
T2	30	28	29	29.00	1.00	Susceptible
T3	26	31	27	28.00	2.65	Susceptible

Table 1 exhibits the level of antibacterial activity of bagras leaf ethanolic extract on *P. aeruginosa*. Previous studies have greatly highlighted the plant's potential for medicinal treatments due to its phytochemical properties, one of which is 1,8-cineole (eucalyptol), which bagras contains 81% percent of (Dihingial et al., 2020). That being said, it has been recognized as one of the contributing factors to bagras' antibacterial activity, which has been effectively demonstrated in these results.

In this study, all treatments (T1, T2, and T3) were classified as susceptible, indicating that the ethanolic extract of bagras leaves effectively inhibited the growth of the bacteria. These findings support the study of Kareem et al. (2020), which also involved the utilization of bagras against *E. coli*, a gram-negative bacterium, resulting in a range of 18 mm, 20.5 mm, and 22.5 mm zones of inhibition. The findings are also akin to that of Hapid et al. (2024), wherein bagras leaves were used against *E. coli*, which led to inhibition zones of 15.67 mm, 17.10 mm, 18.17 mm, and 19.32 mm, respectively. This efficacy also strengthens the findings of Winarti et al. (2024), wherein a mouthwash nanoemulsion combining eucalyptol from bagras and peppermint oil exhibited significant antibacterial activity, with inhibition zones of 13.1 mm against *E. coli* and 17.4 mm against *S. aureus.*

Contrastingly, the findings of this study contradict those of Kambira et al. (2020), who stated that the phytochemical properties found in various species of the *Eucalyptus* genus, including bagras, are not linked to its antibacterial activity, and that Gram-negative bacteria show low susceptibility to such genus. Nevertheless, the satisfactory zones of inhibition shown by each treatment underscores bagras' efficacy as an antibacterial agent, and how potent it can be as a medication.

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Antibacterial Efficacy of the Different Concentrations of Makopa Leaf Ethanolic Extract on the Pseudomonas aeruginosa

The study assessed the effectiveness of the ethanolic extract of makopa leaves in inhibiting the growth of *P. aeruginosa* using three different concentrations: Treatment 4 - 40 mg/mL of makopa leaf ethanolic extract; Treatment 5 - 60 mg/mL of makopa leaf ethanolic extract; and Treatment 6 - 80 mg/mL of makopa leaf ethanolic extract. The researchers determined its antibacterial efficacy by measuring the zone of inhibition per treatment in each replication. Based on this, the researchers obtained the following results.

Table 2. Antibacterial Efficacy of the Different Concentrations of Makopa Leaf Ethanolic Extract on the Pseudomonas aeruginosa

Treatments	Zone of Inhibition (in mm)			Mean	SD	Description
	R1	R2	R3			
T4	0	0	0	0.00	0.00	Resistant
T5	0	0	0	0.00	0.00	Resistant
T6	0	0	0	0.00	0.00	Resistant

Table 2 presents the antibacterial activity of various concentrations of makopa leaf ethanolic extract against *P. aeruginosa*. Previous studies have greatly highlighted the plant's potential for medicinal treatments due to its phytochemical properties, one of which are flavonoids and tannins, which contribute to their antibacterial effects (Choironi & Fareza, 2018). However, the results of this study indicate that none of the tested concentrations exhibited inhibitory effects on *P. aeruginosa*, suggesting that the extract lacks antibacterial efficacy.

The antibacterial activity of makopa leaf ethanolic extract against P. aeruginosa was not observed in this study. Similarly, this finding aligns with the study of Khandaker et al. (2015), which states that makopa ethanolic extract exhibited weak to no inhibitory activity against Gram-negative bacteria, including E. coli, E. cloacae, K. pneumoniae, and S. aureus. Also, the study of Sulthana et al. (2024) found makopa leaf ethanolic extract ineffective as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans exhibited resistance to the said leaf extract.

In contrast, the findings of this study differ from those of Tarigan et al. (2022), who reported that the ethanolic extract of makopa leaves exhibited notable antibacterial activity, particularly against *Bacillus cereus* and *Salmonella enterica*, attributing this effect to the presence of phenolic compounds and tannins in the extract. Similarly, the findings of Yahaya et al. (2023) and Jayakumari et al. (2023) further support the antibacterial potential of ethanolic extracts from makopa leaves, demonstrating significant activity against various pathogenic bacteria, including *E. coli* and *K. pneumoniae*. These varying findings emphasize the need for further research to determine the factors influencing the antibacterial activity of makopa leaf ethanolic extract, such as differences in extraction methods, plant maturity, and bacterial susceptibility.

Antibacterial Efficacy of the Different Concentrations of Bagras and Makopa Leaf Ethanolic Extract on the Pseudomonas aeruginosa

The study investigated the effectiveness of the combination of bagras and makopa leaves ethanolic extract on the growth inhibition of *P. aeruginosa* with three different treatments: Treatment 7 - 40 mg/mL concentration of bagras and makopa leaves ethanolic extract; Treatment 8 - 60 mg/mL concentration of bagras and makopa leaves ethanolic extract; and Treatment 9 - 80 mg/mL concentration of bagras and makopa leaves ethanolic extract. The researchers determined its antibacterial efficacy by measuring the zone of inhibition per treatment in each replication. Hence, the researchers obtained the following results.

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Table 3. Antibacterial Efficacy of the Different Concentrations of Bagras and Makopa Leaf Ethanolic Extract on the Pseudomonas aeruginosa

Treatments	Zone of Inhibition (in mm)			Mean	SD	Description
	R1	R2	R3			
T7	28	34	25	29.00	4.58	Susceptible
T8	25	28	25	26.00	1.73	Susceptible
T9	32	30	32	31.33	1.15	Susceptible

These findings align with the study of Dihingial et al. (2020) and Kareem et al. (2021), which highlights that bagras contains up to 81% eucalyptol (1,8-cineole), a compound known to disrupt bacterial cell membranes, and its antibacterial efficacy in inhibiting Gram-negative bacteria like *E. coli*. To add, makopa is also highly valued for its diverse bioactive compounds, which contributes to its effectiveness in inhibiting the growth of pathogens (Jayakumari et al., 2023; Yahaya et al., 2023). However, conflicting results exist, as Khandaker et al. (2015) and Sulthana et al. (2023) reported weak or inconsistent antibacterial activity against *P. aeruginosa* and *E. coli*, suggesting that makopa's efficacy may depend on the solvent used for extraction.

Hence, these results prove Daniel Moerman's Theory of Non-Random Selection of Medicinal Plants (1979), which suggests that traditional plant use is based on curative properties, with similar taxonomic groups widely utilized. Bagras and makopa, both from the Myrtaceae family, exemplify this by potentially enhancing medicinal effects when combined, supporting the idea that related plants are selected to maximize healing benefits. Overall, the synergistic effect of these compounds increases the antibacterial efficacy of the combined leaf ethanolic extract of bagras and makopa in inhibiting the growth of *P. aeruginosa.*

Antibacterial Efficacy of the Control Group on the *Pseudomonas aeruginosa*

The study included the antibacterial property of the commercial treatment for *P. aeruginosa* using Tobramycin. The researchers determined the antibacterial by measuring the zone of inhibition of Tobramycin in each replication. Hence, the researchers finally attained the following results.

Table 4. Antibacterial Efficacy of the Control Group on the Pseudomonas aeruginosa

Treatments	Zone of Inhibition (in mm)			Mean	SD	Description
	R1	R2	R3			
Tobramycin	52	56	56	54.67	2.31	Susceptible

Table 1 presents the antibacterial property of the tobramycin as the control group against *P. aeruginosa.* This finding aligns with the study of Fiel and Roesch (2022), which states effectiveness of tobramycin in treating infections caused by Gram-negative bacteria, including *P. aeruginosa.* Both studies showed that tobramycin is often used to manage chronic infections like cystic fibrosis, due to its strong antibacterial efficacy. In contrast, the study of Atassi et al. (2023), stated that *P. aeruginosa* exhibits resistance to multiple antibiotics, including aminoglycosides like tobramycin, due to the presence of at least one tobramycin-active gene. It was also reported lower susceptibility rates to tobramycin, ranging from 86% to 88%, showing the difficulty in effectively treating infections caused by *P. aeruginosa*. To conclude, although the result showed tobramycin effective in inhibiting the growth of *P. aeruginosa,* studies have also reported lower susceptibility rates leading to difficulty in treating infections caused by *P. aeruginosa.*

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Significant Difference in the Effectiveness of Different Concentrations of Bagras and Makopa Leaf Ethanolic Extract on the Inhibition of *Pseudomonas aeruginosa*

Table 3 shows the results of one-way analysis of variance to determine the significance of the difference in the effectiveness of different concentrations of bagras and makopa leaf ethanolic extract on the inhibition of *P. aeruginosa* growth. It can be observed that the F value is 95.033 with 8 and 18 degrees of freedom. The p-value is 0.000 which is less than 0.05. This further means that the null hypothesis should be rejected, indicating that at least one among the nine treatments significantly differ from the other in terms of its effectiveness in the inhibition of *P. aeruginosa* growth.

Table 5. Significant Difference in the Effectiveness of Different Concentrations of Bagras and Makopa Leaf Ethanolic Extract on the Inhibition of Pseudomonas aeruginosa

	Sum of Squares	**df**	**Mean Square**	**F**	**p**	**Decision**
Between Groups	4871.333	8	608.917	95.033	0.000	Reject H_0
Within Groups	115.333	18	6.407			*(*Significant)
Total	4986.667	26				

To determine which among the nine concentrations significantly differ from the other, post hoc analysis was conducted particularly the pair-wise comparisons of sample means via the Tukey HSD test. The Tukey's honestly significant difference test (Tukey's HSD) is used to test differences among sample means for significance. The Tukey's HSD test is one of several tests designed for this purpose and fully controls this Type I error rate (Nanda et al., 2021).

Meanwhile, Table 4 presents the results of the post hoc comparisons conducted using the Tukey HSD test. This analysis was performed to identify which of the nine treatments showed significant differences in effectiveness. The results reveal that significant differences were found between Treatments 1, 2, 3, 7, 8, and 9 compared to Treatments 4, 5, and 6, as shown by the p-values less than 0.05. Additionally, positive mean differences between these treatments indicate that the former treatments are significantly more effective than the latter. This suggests that Treatments 4, 5, and 6 were not effective in inhibiting the growth of *P. aeruginosa*.

Table 6. Post Hoc Comparisons using the Tukey HSD Test

	Mean Difference	**p**	**Decision**	**Interpretation**
Between T1 and T2	-2.333	0.962	Fail to Reject H_0	Not Significant
Between T1 and T3	-1.333	0.999	Fail to Reject H_0	Not Significant
Between T1 and T4	26.667	0.000	Reject H_0	Significant
Between T1 and T5	26.667	0.000	Reject H_0	Significant
Between T1 and T6	26.667	0.000	Reject H_0	Significant
Between T1 and T7	-2.333	0.962	Fail to Reject H_0	Not Significant
Between T1 and T8	0.667	1.000	Fail to Reject H_0	Not Significant
Between T1 and T9	-4.667	0.413	Fail to Reject H_0	Not Significant

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Between T2 and T3	1.000	1.000	Fail to Reject H_0	Not Significant
Between T2 and T4	29.000	0.000	Reject H_0	Significant
Between T2 and T5	29.000	0.000	Reject H_0	Significant
Between T2 and T6	29.000	0.000	Reject H_0	Significant
Between T2 and T7	0.000	1.000	Fail to Reject H_0	Not Significant
Between T2 and T8	3.000	0.863	Fail to Reject H_0	Not Significant
Between T2 and T9	-2.333	0.962	Fail to Reject H_0	Not Significant
Between T3 and T4	28.000	0.000	Reject H_0	Significant
Between T3 and T5	28.000	0.000	Reject H_0	Significant
Between T3 and T6	28.000	0.000	Reject H_0	Significant
Between T3 and T7	-1.000	1.000	Fail to Reject H_0	Not Significant
Between T3 and T8	2.000	0.984	Fail to Reject H_0	Not Significant
Between T3 and T9	-3.333	0.787	Fail to Reject H_0	Not Significant
Between T4 and T5	0.000	1.000	Fail to Reject H_0	Not Significant
Between T4 and T6	0.000	1.000	Fail to Reject H_0	Not Significant
Between T4 and T7	-29.000	0.000	Reject H_0	Significant
Between T4 and T8	-26.000	0.000	Reject H_0	Significant
Between T4 and T9	-31.333	0.000	Reject H_0	Significant
Between T5 and T6	0.000	1.000	Fail to Reject H_0	Not Significant
Between T5 and T7	-29.000	0.000	Reject H_0	Significant
Between T5 and T8	-26.000	0.000	Reject H_0	Significant
Between T5 and T9	-31.333	0.000	Reject H_0	Significant
Between T6 and T7	-29.000	0.000	Reject H_0	Significant
Between T6 and T8	-26.000	0.000	Reject H_0	Significant
Between T6 and T9	-31.333	0.000	Reject H_0	Significant
Between T7 and T8	3.000	0.863	Fail to Reject H_0	Not Significant
Between T7 and T9	-2.333	0.962	Fail to Reject H_0	Not Significant
Between T8 and T9	-5.333	0.260	Fail to Reject H_0	Not Significant

Based on the test and the mean of each treatment, T1, T2, T3, T7, T8, and T9 are considered to be significantly more effective in inhibiting the growth of *P. aeruginosa.* This means that out of the various leaf ethanolic

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extracts tested, the different concentrations of the leaf ethanolic extract with the presence of bagras showed more efficacy compared to the leaf ethanolic extract consisting of just makopa. These findings align with the study of Dihingial et al. (2020) with regards to bagras, he stated in the review that eucalyptus species, and *E. deglupta* Blume in particular, has an 81% 1,8-cineole (eucalyptol) content, making it effectively antibacterial due to its ability to disrupt cell membranes and alter their permeability. Additionally, Galan et al. (2020), also highlighted the potential pharmacological benefits of eucalyptol, which exhibits antibacterial properties beneficial for respiratory health. Aforementioned studies suggest that bagras showed effectiveness in inhibiting the growth of *P. aeruginosa* because of its antibacterial content compared to the other plant used.

On the other hand, T7, T8, and T9 the combination of ethanolic extract of both the bagras and makopa leaves also showed effectiveness in inhibiting the growth of *P. aeruginosa.* Phytochemicals such as flavonoids and phenolic compounds are known to be present in both bagras and makopa, this shows the synergistic antibacterial effects when combining plants. The following studies from Tarigan et al. (2021) showed that silver nanoparticles (AgNPs) synthesized from the bagras extract provide valuable insights into their phytochemical properties and health benefits. It shows that various phytochemicals, including flavonoids and phenolic compounds, are crucial for the biosynthesis and stabilization of AgNPs, which boosts their antibacterial effectiveness against Gram-positive and Gram-negative bacteria. Also, the study of Issah Yahay et al. (2023) stated that makopa leaves contain compounds, including flavonoids, phenolic compounds, alkaloids, and saponins, which enhances medical and therapeutic potential as an antibacterial agent. These findings highlights the potential of enhanced antibacterial efficacy in terms of combining plants that share the same bioactive compounds.

Significant Difference in the Effectiveness Between Bagras and Makopa Treatment and Control Treatment on the Inhibition of *Pseudomonas aeruginosa*

Table 3 presents the results of a one-way ANOVA to assess the significance of differences in the effectiveness of various concentrations of bagras and makopa leaf ethanolic extracts on inhibiting *P. aeruginosa*. The F value is 13.24, with 4 and 10 degrees of freedom. The p-value is 0.000, which is less than 0.05. This indicates that the null hypothesis should be rejected, meaning that at least one of the six treatments (T1, T2, T3, T7, T8, T9) or the control group treatment differs significantly from the others in terms of its effectiveness in inhibiting *P. aeruginosa*.

Table 7. Significant Difference in the Effectiveness of Different Concentrations of Bagras and Makopa Leaf Extract Extract on the Inhibition of Pseudomonas aeruginosa

	Sum of Squares	**df**	**Mean Square**	**F**	**p**	**Decision**
Between Groups	1837.810	6	306.302	34.034	0.000	Reject H_0
Within Groups	126.000	14	9.000			*(Significant)*
Total	1963.810	20				

Post hoc comparisons using the Tukey HSD test reveal a significant difference in the effectiveness between Treatments 1, 2, 3, 7, 8, 9, and the control group, as indicated by the p-values less than 0.05. The results also show negative mean differences between these treatments, suggesting that the control group is significantly more effective in inhibiting the growth of *P. aeruginosa* compared to the bagras and makopa leaf ethanolic extract treatments.

Table 8. Post Hoc Comparison on T1, T2, T3, T7, T8, T9, and Control using the Tukey HSD Test

	Mean Difference	**p**	**Decision**	**Interpretation**
Between T1 and Control	-28.000	0.000	Reject H_0	Significant

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Between T2 and Control	-25.667	0.000	Reject H_0	Significant
Between T3 and Control	-26.667	0.000	Reject H_0	Significant
Between T7 and Control	-25.667	0.000	Reject H_0	Significant
Between T8 and Control	-28.667	0.000	Reject H_0	Significant
Between T9 and Control	-23.333	0.000	Reject H_0	Significant

Overall, the Tukey HSD post-hoc test illuminated statistically significant differences between the various treatments (T1, T2, T3, T7, T8, and T9) and the control group in their effectiveness in inhibiting the growth of *P. aeruginosa*. As indicated by the data, the results showed that all treatments had a significant impact, as the null hypothesis was rejected in every comparison. This means that each treatment had a distinct effect compared to the baseline established by the control group. Although the control treatment, tobramycin, had greater effectiveness in inhibiting the growth of *P. aeruginosa*, the plant extracts also demonstrated considerable inhibitory effects. These results support the study of Kaur et al. (2018) in which the crude extract of bagras leaves is rich in phytochemicals, including phenolics and flavonoids.

Furthermore, the antibacterial potential of phytochemicals derived from the bagras plant is evident in the substantial zones of inhibition observed after treatment. These results align with previous studies of Hapid et al. (2024), and Galan et al. (2020), which identified the presence of chemical components, including α-phellandrene, α-pinene, and p-cymene. Additionally, Issah Yahay et al. (2023) highlighted the significance of the makopa plant, noting that its leaves contain various bioactive compounds, including flavonoids, phenolic compounds, alkaloids, and saponins, which support its medical and therapeutic potential as an antibacterial agent. The synergistic combination of bagras and makopa demonstrated a noticeably greater zone of inhibition compared to when each plant extract was used on its own. This suggests that their combined bioactive compounds enhanced antibacterial effectiveness.

SUMMARY

This study aimed to evaluate the antibacterial efficacy of bagras (*Eucalyptus deglupta* Blume) and makopa (*Syzygium samarangense* [Blume] Merr. & L.M. Perry) leaf ethanolic extract at varying concentrations in inhibiting the growth of *P. aeruginosa*. The findings revealed that the bagras leaf ethanolic extract demonstrated strong antibacterial activity across all concentrations, with zones of inhibition ranging from 21mm to 31mm. On the other hand, makopa leaf extract was completely ineffective alone, as no zones of inhibition were observed at any concentration.

Notably, the combination of bagras and makopa extracts produced better antibacterial results than the bagras leaf ethanolic extract alone. Results showed large zones of inhibition (ranging from 25 mm to 34 mm), indicating possible synergistic effects between the two extracts. Compared to the commercial antibiotic Tobramycin, the bagras leaf ethanolic extract and the combination of both makopa and bagras showed significant differences in antibacterial efficacy, highlighting its potential as a natural antibacterial agent. The ineffectiveness of the makopa leaf ethanolic extract also emphasizes the value of exploring extract combinations.

CONCLUSION

Acknowledging the need for effective alternatives to conventional antibiotics in inhibiting the growth of *P. aeruginosa*, this research explored the potential of ethanolic extracts from bagras and makopa leaves as antibacterial agents. The study aimed to assess the antibacterial properties of these plant extracts and their effectiveness in inhibiting bacterial growth. The findings of this study yielded the following conclusions:

1. The ethanolic extract of bagras leaves exhibited high antibacterial activity against *P. aeruginosa* at all tested concentrations, indicating strong susceptibility. This suggests its potential as an alternative

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treatment to antibiotics for combating *P. aeruginosa* infections.

2. The ethanolic extract of makopa leaves exhibited no antibacterial activity against *P. aeruginosa* at any tested concentration, as evidenced by the absence of inhibition zones. This indicates that the extract lacks antibacterial efficacy, regardless of concentration. These findings indicate that makopa leaves may not contain bioactive compounds effective against *P. aeruginosa*, emphasizing the need for further research to investigate other plant extracts or alternative extraction methods that could enhance antibacterial properties.
3. The combined bagras and makopa leaf ethanolic extracts exhibited improved antibacterial efficacy against *P. aeruginosa*, even though makopa extract alone showed no inhibitory effect. The interaction between the compounds in both extracts resulted in greater antibacterial activity compared to the bagras leaf ethanolic extract alone. While makopa extract may not have direct antibacterial properties, it may contain compounds that enhance the effectiveness of bagras extract. These findings indicate that plant extracts with limited individual antibacterial activity may still contribute to improving the antibacterial efficacy of other plants when combined.
4. The commercial antibiotic tobramycin exhibited strong antibacterial activity against *Pseudomonas aeruginosa*, as indicated by significant zones of inhibition. This confirms its effectiveness in inhibiting *P. aeruginosa* growth, supporting its role as a widely used antibiotic.
5. The different concentrations of the ethanolic extract of bagras and makopa as compared to the control treatment (T10) showed negative mean differences in inhibiting the growth of *P. aeruginosa*. This result implies that the control treatment exhibited significant effectiveness in inhibiting the growth of *P. eruginosa* compared to the bagras and makopa leaf ethanolic extract.

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