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Antibacterial Potential of Batuan (*Garcinia binucao*) Fruit Crude Extract Against *Staphylococcus aureus*: An in Vitro Study Employing Agar Disc Diffusion Assay

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ABSTRACT

This study investigated the in vitro antibacterial activity of Batuan (*Garcinia binucao*) fruit crude extract against *Staphylococcus aureus*, a bacterium commonly associated with skin infections and colonization of the mouth and nose. A parallel-group experimental design was employed, testing three concentrations of the extract (50%, 75%, and 100%) using the agar disk diffusion method. The fruit samples underwent authentication, extraction, dilution, and antibacterial screening, with each treatment replicated in three trials. The results revealed that the 100% concentration exhibited the largest inhibition zone (29.91 mm on average), followed by 75% (21.75 mm), oxacillin (21.70 mm), and 50% (17.60 mm). The statistical analysis using one-way ANOVA indicated a significant difference among the treatments, with the computed F-value (32.9290) exceeding the critical F-value (4.0662), thereby rejecting the null hypothesis. These findings demonstrate that antibacterial activity increases with extract concentration, confirming that Batuan fruit crude extract is effective against *Staphylococcus aureus* and has potential as a natural antimicrobial agent.

Keywords: *Batuan, Staphylococcus aureus, Abscesses, Crude, Extract.*

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CHAPTER ONE
INTRODUCTION

One of the biggest dangers to global development and public health is antimicrobial resistance (AMR). Bacterial AMR is thought to have contributed to 4.95 million fatalities worldwide in 2019 and been directly responsible for 1.27 million deaths (World Health Organization: WHO, 2023).

The development of new natural antimicrobial agents is crucial due to the rapid increase in antibiotic-resistant microorganisms, especially *Staphylococcus aureus*. The bacteria, *Staphylococcus aureus* are the most common cause of skin infections. This may result in skin redness, blisters, and boils. These infections are common around the mouth and nose, but if the infection occurs people can develop pneumonia and other breathing problems from the abscesses that can form. *Staphylococcus aureus* can also damage the heart valves and lead to heart failure (Staph Infection, 2025).

Consequently, multiple studies evaluated plant extracts to be used as antimicrobial, antiparasitic, and antibacterial cure for different kinds of disease or infection. An unfamiliar plant species, Batuan (*Garcinia binucao*) is native to the Philippines and found across the country, particularly in the islands of Luzon and Visayas (Tantengco et al. 2018 quoted in the study of Teresa et al., 2024). Batuan is a species of the *Garcinia* endemic that contains Polyphenols that could kill the antibiotic resistant bacteria, *Staphylococcus aureus*. According to the Phytochemical screening of the selected indigenous fruits in the Philippines, total phenolics were the highest in batuan with 758 mg gallic acid equivalent (GAE) and 100g fresh matter (FM) (Recuenco et. al, 2020).

The prevalence of antibiotic-resistant strains, such as MRSA (methicillin-resistant *Staphylococcus aureus*), has made this study more pertinent. Furthermore, a study by Zhang et al. (2023) focused only on the structure–activity relationship of xanthones from *Garcinia* species. Their study discovered that prenylated xanthones such as α-mangostin (*Garcinia mangostana)* had potent antibacterial against *Staphylococcus aureus* with minimum inhibitory concentration (MIC) = 1 μg/ml. It does so by the destruction of bacterial membrane, disruption of efflux pumps and DNA processes of bacteria, which ultimately weakens the defense mechanism of the pathogen.

Expanding on this, Felix et al. (2022) the bactericidal and anti-biofilm effect of α-mangostin on *Staphylococcus aureus* persister cells was investigated. Their findings showed that the substance was able to eliminate 99.99% of persister cells within 30 minutes and also disrupted biofilms. Furthermore, α-mangostin also decreased the expression of resistance (norA, norB) and the gene harbored for heat stress (dnaK), thus being a very efficient agent also for dormant and biofilm bacteria. Locally, these potent flavonoid/xanthone compounds in *Garcinia binucao* were tested positive for these compounds as verified by Recuenco et al. (2020) to have a phytochemical screening and the selected Philippine fruits. Their results indicated that *Garcinia binucao* exhibited the highest amount of TP (758 mg GAE/100 g) compared to other fruits analyzed and it is associated with their high antioxidant and antimicrobial activities. These polyphenolics, in particular flavonoids and xanthones, are thought to be responsible for its bioactivity.

In addition, supporting the therapeutic potential of these compounds, Creer (2022) also investigated the anti-inflammatory and cytotoxic properties of *Garcinia binucao* fruit extract. The extract had a moderate anti-inflammatory activity and low cytotoxicity at lower concentrations using the brine shrimp lethality assay and protein denaturation test. This indicates that not only might it be antibacterial, but it might be able to tamp down inflammation, a good world to inhabit if you're facing infectious agents like *Staphylococcus aureus* that create tissue damage and swelling.

Terpenes in addition to flavonoids and xanthones have been reported to be potent antimicrobial agents due to their mode of action in quorum sensing inhibition and disruption of the bacterial membrane. Salinas et al. (2022) studied ternary combinations between terpenes (linalool and trans-caryophyllene) and their effects in *Staphylococcus aureus* biofilm. Their research exhibited 88% inhibition of biofilm formation, as well as the suppression of virulence genes (sdrD, spa, agr). These results demonstrate that terpenes not only limit the growth of bacteria, but also inhibit them from attaching and persisting, a critical attribute in chronic infections.

While the previous study is directed at purified terpene compounds, *Garcinia binucao* contains terpenes in itself, demonstrated Also by Adolacion et al. (2023) that has tested the safety of its methanolic fruit extract by Allium cepa root tip methodology. Though the extract was found to have a mild genotoxic effect at high concentrations (24.5% mitotic index reduction), this research supports the notion that *Garcinia binucao* bioactive constituents (terpenes included) are highly potent and should be applied at controlled concentrations in antibacterial uses.

The study of Meena et al., 2022 have shown that bioactivity of certain plant extracts does not lose its effect. The bioactivity of certain plant extracts does not lose its substantially over time under appropriate storage and environmental conditions. For example, in the work on *Thevetia peruviana* leaf extracts, both crude extract and formulated herbal mixtures retained antifungal activity against *Alternaria solani* after 6 and 12 months of storage, with minimum inhibitory concentration (MIC) values comparable to those of freshly prepared extracts. These observations indicate that, given correct formulation and storage, plant extracts can preserve

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their antimicrobial or antifungal capabilities, and more broadly, biological activities over long durations supporting their use in applications where shelf-life is important without sacrificing potency. This study was essential in the development of the batuan crude extracts with varying concentrations, as well as in emphasizing the importance of proper storage practices to ensure their long-term stability and efficacy for practical applications.

In addition, the antimicrobial effect of these compounds was also analyzed by Calica et al. (2024), who examined the antibacterial activities of *Garcinia binucao* against the multidrug resistant *Acinetobacter baumannii,* a Gram-negative bacterium. "The frugivore bactericidal effect was similar to the one of the antibiotic amikacin. While *A. baumannii* is not the sort of *Staphylococcus aureus*, both develop resistance, and the extract's activity against resistant pathogen also supports its broad range in antimicrobial therapy, especially when common phytochemical contents such as terpenes and flavonoids are taken into the account.

Together, these findings make a strong support for the antibacterial, anti-biofilm and therapeutic activity of *Garcinia binucao* fruit extract. The active compounds such as flavonoids, xanthones and terpenes showed bioactivity based on depicting local and international study. Ranging from direct bactericidal effects to biofilm prevention or anti-inflammatory activity, these classes of phytochemicals correspond well to the challenges of current trends in antimicrobial research. The present investigation established, in vitro antimicrobial assessment of *Garcinia binucao* extract against *Staphylococcus aureus* amongst the increasing evidence of plant treatment of bacterial infection and resistance.

Furthermore, this study included a comparison with commercially available antibiotics, specifically, Oxacillin. Oxacillin, a (beta) β-lactam antibiotic belonging to the penicillin class, is commonly utilized for the treatment of bacterial infections caused by susceptible, predominantly gram-positive organisms. In this study, oxacillin was employed as the control group.

The Traditional and Alternative Medicine Act (TAMA) of 1997, also known as Republic Act No. 8423, serves as the foundation for this study since it declares in Section 12 of the Traditional and Alternative Health Care Advocacy and Research Program that the Institute will launch a national campaign to increase support for the Act's goals. The law further stipulated that the Institute must use scientific research approaches to develop and carry out a research program on the indigenous traditional health care practices by "traditional healers" in the Philippines (FAOLEX, 2022).

Although this research sets the antibacterial activity of Batuan (*Garcinia binucao*) fruit crude extract against *Staphylococcus aureus*, more studies are lacking on its efficacy against more intricate microbial strains causing skin infections and pneumonia, its synergy when blended with standard antibiotics, as well as its safety through cytotoxicity assays in human cell lines. Filling in these gaps would more solidify and support the extract's potential as a natural alternative antibacterial agent.

This study has limitations that may affect the interpretation of the results. Firstly, the experiment was conducted solely through in vitro testing using the agar disk diffusion method, which does not account for in vivo factors such as toxicity, metabolism, or interactions within a living system. Another limitation is that only *Staphylococcus aureus* was tested, which narrows the scope of antibacterial activity and does not represent the extract's potential effects on other pathogenic bacteria. Time and resource constraints restricted the study to laboratory-scale testing, preventing wider validation across different laboratories or testing environments. Despite these limitations, the study provides initial insights into the antibacterial potential of Batuan (*Garcinia binucao*) fruit crude extract against *Staphylococcus aureus*.

The result of this study is advantageous in the field of medicine because of its importance as an alternative and natural treatment against *Staphylococcus aureus.* The outcome of the study is also favorable to students or future researchers, professionals, batuan farmers, and even the community because it will provide new concepts and create new opportunities and also create new ideas in the field of research and medicine.

This study aimed to determine the effectiveness of Batuan fruit crude extract against *Staphylococcus aureus*. It was quantitatively measured by the measurement of the zone of inhibition through the agar disk diffusion method. More specifically, it determined the level of the antibacterial activity of three different concentrations of Batuan fruit crude extract as measured through the zones of inhibition. It also identified which concentration of the extract possesses the highest antibacterial activity against *Staphylococcus aureus*. This provides information regarding its viability as a natural antibacterial agent.

This study investigated the antibacterial activity of the three concentrations (50%, 75% and 100%) in comparison to the commercial antibiotic, Oxacillin against *Staphylococcus aureus* and aims to specifically answer the following questions:

- What is the antibacterial activity of Batuan fruit crude extract against *Staphylococcus aureus* based on the zone of inhibition observed in the Agar disk diffusion method?
- Which concentration of Batuan fruit crude extract shows the highest antibacterial activity against *Staphylococcus aureus*?
- What is the significant difference between the antibacterial activity of Batuan fruit crude extract and oxacillin in terms of zone of inhibition?

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- **H_0**: There is no significant difference in the antibacterial activity among the three concentrations of Batuan fruit crude extracts and oxacillin in terms of zone of inhibition.

➢ *Theoretical Framework*

This research is anchored with two foundational theories: Germ's Theory of Disease and Koch's Postulate.

Germ Theory of Disease is a theory proposed by Louis Pasteur and Robert Koch in the late 1900s that was later cited in the study of the Biology Insights Team (2025). The theory states that microorganisms are responsible for most diseases. Louis Pasteur and Robert Koch established this theory that transformed the field of medical science. Research approach of Louis Pasteur and Robert Koch in the late 1900s showed that a single pathogen such as *Staphylococcus aureus* and other natural bacteria and microorganisms found in our ecosystem that later forms into a large group of organisms that results in the form of several known diseases. (Scannapieco et al., 2024) This allowed for new leaps in proper methods and protocols in both hygiene and sterilization, as well as the creations of antibiotics, fundamentally changing health and clinical medical practices among health facilities.

This theory of both Pasteur and Koch plays a crucial role in this study, as it theorizes that microorganisms such as *Staphylococcus aureus* is an infection that can eventually cause pneumonia. As this theory discussed that microorganisms such as *Staphylococcus aureus* starts within a singular bacterial form and multiplies tremendously, thus forming into severe diseases known today. This information synthesize that an effective antibacterial agent can control and treat such pathogens through determining the antibacterial activity of Batuan (*Garcinia binucao*).

Koch's Postulates are theories created by Robert Koch in 1884 that was later cited in the journal of Hou et al., 2023. It states that microorganisms are the main cause of serious diseases known today. The theories outlined in Koch's Postulates follow a sequence of conditions developed to find the correlation of a specific microorganism with its specific product of disease. Late in the 19th century, he described a set of four criteria designed to prove the relationship between a specific microorganism and a named disease. *Staphylococcus aureus* is a gram-positive, lacet-shaped bacterium that quickly forms into community-acquired pneumonia (Dion et al., 2023). These theories have been used historically to support the claim that specific bacteria cause certain diseases, thus providing a scientific basis for understanding infections. This theory is crucial to the study because it provides a systematic way of proving that *Staphylococcus aureus* is the causative agent of pneumonia and to test the antibacterial property of Batuan (*Garcinia binucao*) fruit crude extract. In line with these assumptions, the study can be capable of culturing *Staphylococcus aureus*, analyzing its in vitro growth, testing the application of the extract to determine its inhibitory activities, and confirming the decrease in bacterial density.

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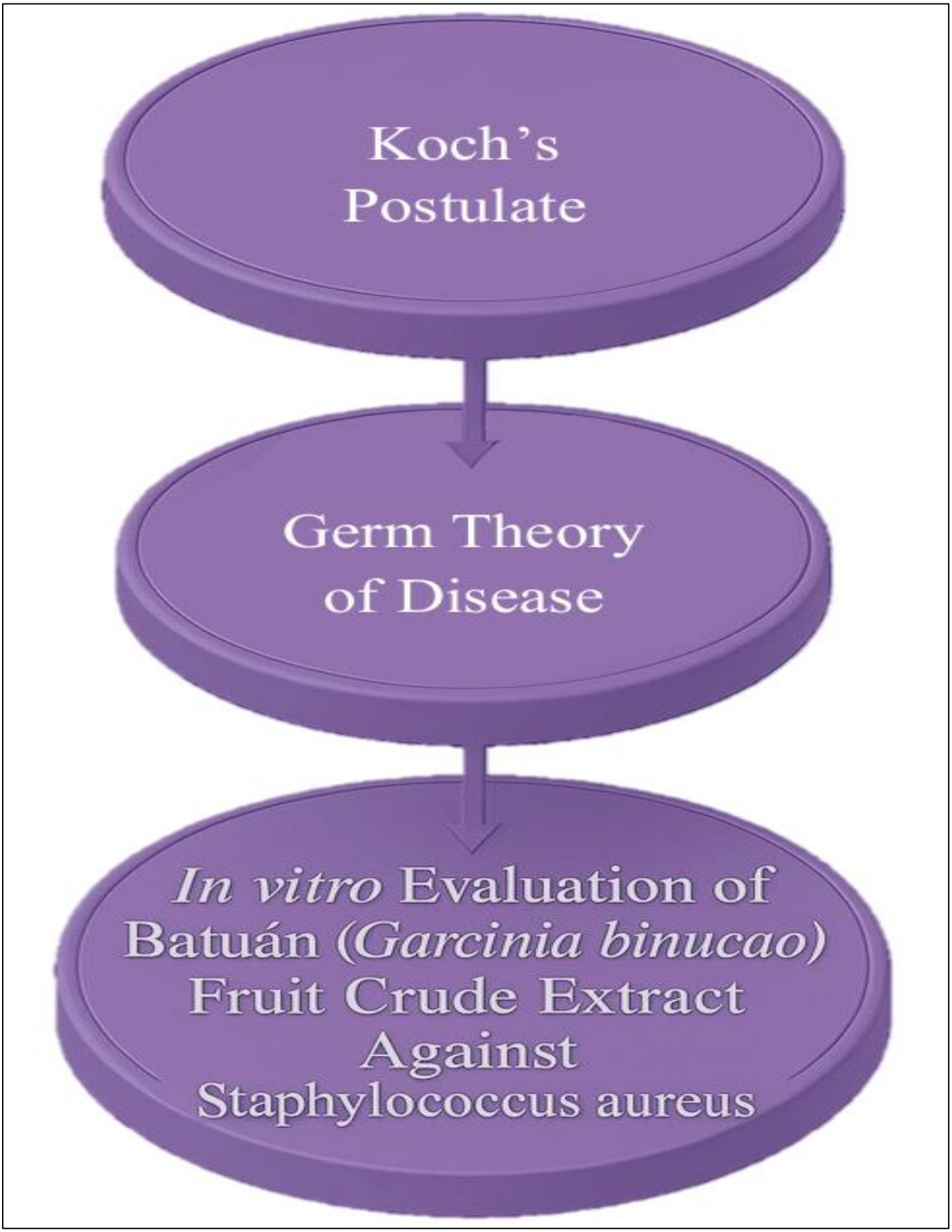

Fig 1 Theoretical Paradigm

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CHAPTER TWO
METHODOLOGY

This study used an Experimental Research Design, specifically the Parallel Group Design (PGD), where the subject (*S. aureus*) is exposed to the treatments (50%,75%,100%, and Oxacillin 1ug) and measured after intervention. Parallel group design is an experimental study where participants are randomly assigned to one of two or more independent treatment groups. Each group receives a different treatment throughout the study (Turner, 2020). With this design, the researchers were able to compare the effects of the different treatments simultaneously.

Four groups were formed: three treatment groups with the Batuan fruit crude extract concentrations of 50%, 75%, and 100% and one control group receiving Oxacillin, the reference standard antibiotic and the bacterial colony *S. aureus* served as the subject that was treated. It is particularly appropriate to use a parallel group design in this research. Antibacterial activity was measured using the agar disc diffusion method, focusing on the extract's potential along with the commercial antibiotic specifically oxacillin.

Table 1 Presentation of Variables and Treatments

	Experimental Treatments			
	Treatment 1	**Treatment 2**	**Treatment 3**	**Control Group**
Independent Variable	50% Batuan fruit crude extract	75% Batuan fruit crude extract	100% Batuan fruit crude extract	Oxacillin 1 ug
Dependent Variable	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*

➢ *Research Procedure*

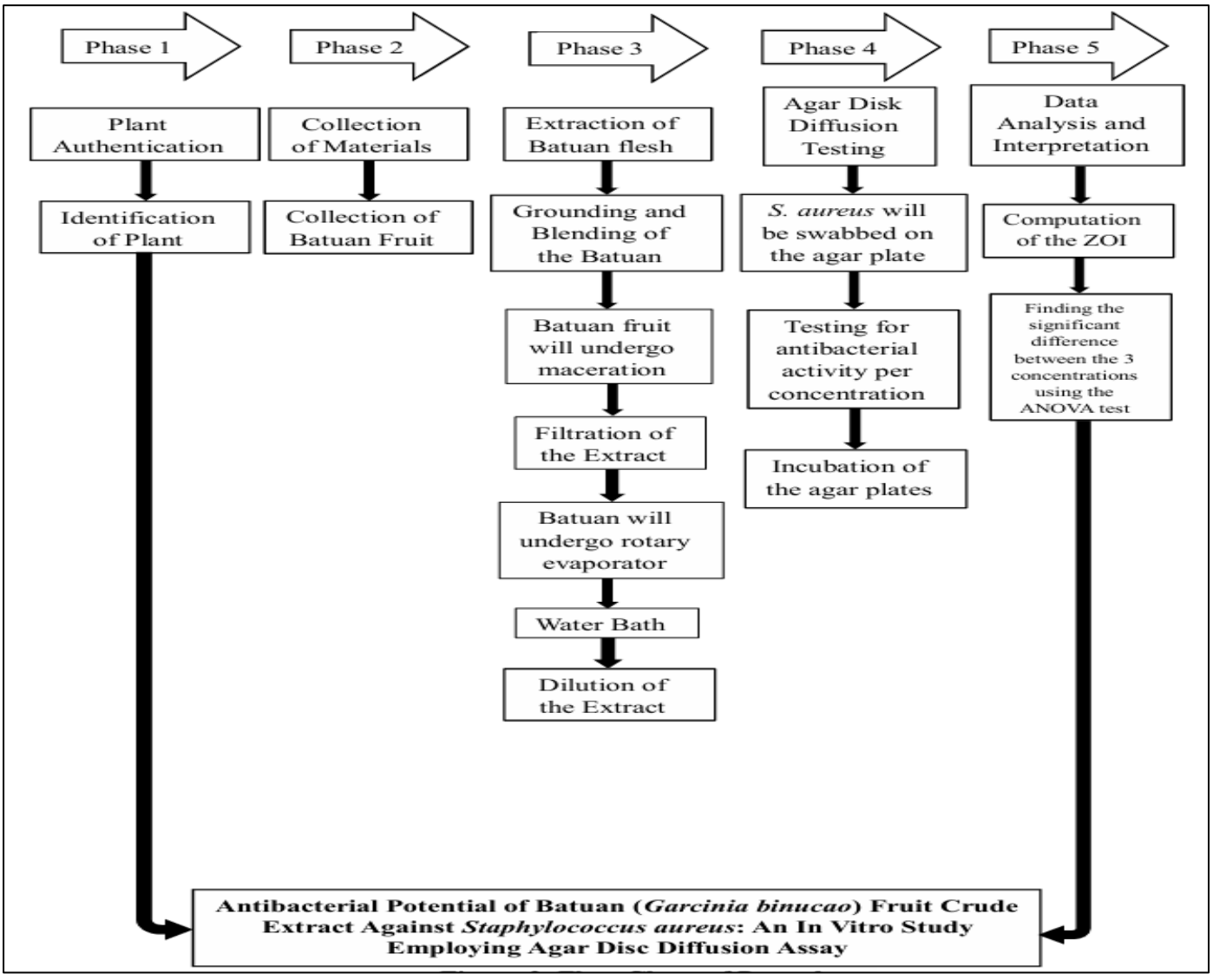

Fig 2 Flow Chart of Procedures

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The materials used in this study was Batuan (*Garcinia binucao*), which was provided by the researchers by collecting it from Batuan trees. The DOST-ITDI also used the microorganism *S. aureus* and the control variable, Oxacillin. The laboratory equipment such as mortar and pestle, blender, filtration set up, solvent container, and the water bath which were used during the fruit extraction process were provided by the extraction facility.

The Batuan (*Garcinia binucao*) fruit were freshly collected and authenticated through a morphological identification method called Phyllotaxy, which observes the arrangement of leaves on the plant stem. This verification step ensured the correct botanical identity of the fruit. In May 19, 2025, the collected specimen was submitted to the Provincial Environment and Natural Resources Office (PENRO) for formal plant identification. Ethical collection practices were observed prior to experimental use.

Approximately 2.815 kilograms of fresh Batuan fruit were harvested in May 25, 2025, from Purok 4, Barangay Lugui, Labo, Camarines Norte. The collection was done manually, ensuring the fruits are mature and free from damage. The fruits were placed in sterilized containers and stored in cool, shaded conditions during transport. In May 28, 2025, one of the researchers accompanied with their parent delivered the samples to Green Matter Botanicals in Taguig City for extraction.

The extraction of the Batuan (*Garcinia binucao*) fruit crude extract was done through a series of procedures. This procedure was conducted by Green Matter Botanicals, a certified facility specializing in plant-based extractions. A total of 2.815 kg of fresh Batuan (*Garcinia binucao*) fruits was washed, peeled, and manually blended into smaller pieces to facilitate solvent penetration. The blended material underwent maceration in 6 liters of 95% ethanol at room temperature for 48 hours, with occasional manual stirring to enhance extraction efficiency. After maceration, the mixture was filtered to remove plant residues. The ethanol filtrate was concentrated using a rotary evaporator, followed by further evaporation in a 60°C water bath until a semi-solid crude extract was obtained. The resulting extract was transferred into amber glass containers to protect it from light-induced degradation and it was stored under refrigerated conditions (2–8°C) to preserve its phytochemical stability. The final expected yield is approximately 133.9 grams of fruit crude extract.

➢ *Experimental Process*

The fruit crude extract of the Batuan underwent Agar Disk Diffusion Test. The Antibacterial Activity Testing was done in DOST-ITDI in Taguig City, Metro Manila, Philippines. The testing facility received the extracts on July 16, 2025, was tested on July 29 until July 30, 2025 and the researchers received the test results on August 6, 2025 via E-mail.

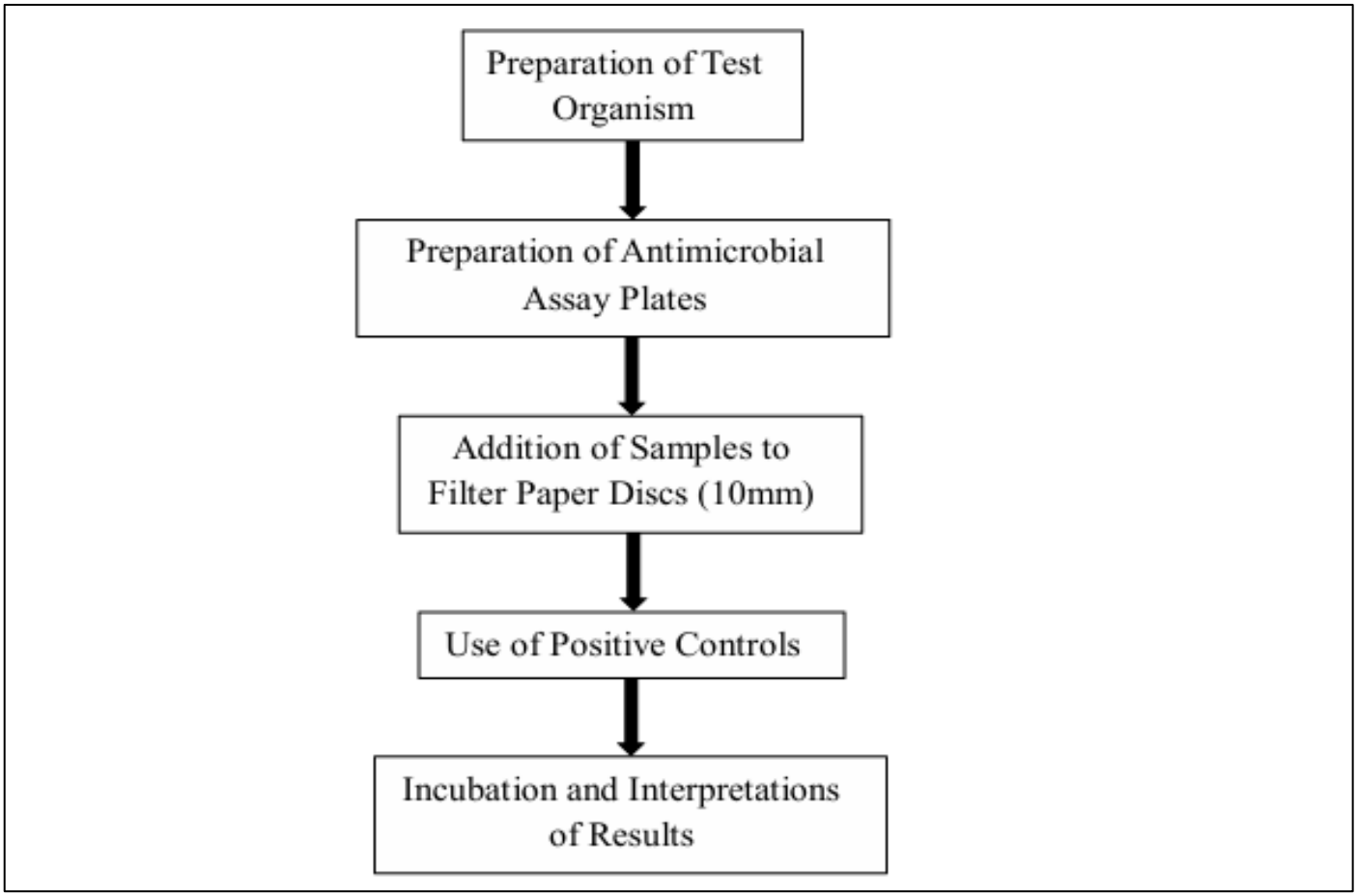

Fig 3 Experimental Layout

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For the *S. aureus*, it was swabbed on agar surface for bacteria, sterile filter paper disk containing the different concentrations of the fruit crude extract (50%, 75% and 100%) was placed. The significance difference of antibacterial activity was evaluated by measuring the Zone of Inhibition (ZOI) in millimeters (mm). Ethanol and flame were used to sterilize all tools and materials that come into contact with the bacteria to keep conditions clean. The *S. aureus* was first prepared by inoculating the bacterial isolate to 10ml Tryptic Soy Broth (TSB) and was incubated overnight at 35°C.

For the preparation of Antimicrobial Assay Plates, the overnight culture was adjusted to a turbidity comparable to 0.5 McFarland Standard first before adding 200uL of the adjusted bacterial suspension to a standard-size sterile petri dish. An approximate 15ml-20ml of Mueller-Hinton Agar was added and swirled onto the plates before being allowed to coagulate and dry.

The three plates were incubated at 35°C for an hour before adding the filter paper discs (10mm) with samples. Given that the fruit crude extracts were thick and viscous, the pipette was not used; instead, the papers discs were dipped in the extracts (50%, 75%, 100%) and the control group and were allowed to soak into the paper same process with the control group oxacillin.

The excess extracts were removed by tapping into the sides of the container, and the discs were added to the previously prepared assay plates. The three plates were then inverted and incubated at 35°C for an entire night. After the incubation, zone of inhibition (ZOI) was observed and measured in millimeters (mm) using a caliper.

The One-way ANOVA (Analysis of Variance) test was used in this study. Comparing the means of two or more groups for one dependent variable (Ross & Willson, 2017). ANOVA was used to evaluate whether there was a statistically significant difference among the three concentrations of Batuan (*Garcinia binucao*) fruit crude extract and oxacillin in terms of the mean zone of inhibition.

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CHAPTER THREE
RESULTS AND DISCUSSION

Table 2 Radical Scavenging Values of Fruit Samples from the Study of Recuenco et al., (2022)

Sample	% Of moisture	Total phenolics* (mg GAE / 100 g FM)	Total flavonoids* (mg CE / 100g FM)	Radical scavenging* (mg AEAC / 100 g FM)	Ferric reducing power * (mg AAE / 100 g FM)	pH of extract	*E. coli* MIC_{50} (mg GAE / mL)	*S. aureus* MIC_{50} (mg GAE / mL)
Ficus ulmifolia	71.1 ± 0.7	608 ± 22a	362 ± 7a	482.7 ± 4.2a	97.3 ± 3.5a	5	8.13	12.88
Antidesma. bunius	83.8 ± 0.4	278 ± 10b	161 ± 7b	361.0 ± 5.8a	44.8 ± 1.6b	6	2.00	3.00
Garcinia binucao	**84.1 ± 0.6**	**758 ± 22c**	**312 ± 9c**	**479.7 ± 4.0a**	**121.1 ± 3.5c**	**3**	**2.00**	**2.00**
Artocarpus altilis	77.6 ± 0.3	234 ± 5d	132 ± 7d	396.4 ± 5.9c	37.0 ± 1.0b	3	1.70	3.50
Citrus hystrix	83.6 ± 0.3	403 ± 10e	221 ± 5e	359.7 ± 3.2a	84.9 ± 5.0d	3	1.70	3.00
Ficus Pseudopalma	76.4 ± 0.4	530 ± 3e	421 ± 5e	439.3 ± 7.0b	89.4 ± 1.5d	3	1.70	3.00
Mangifera altissima	86.2 ± 0.2	408 ± 6f	283 ± 6f	289.0 ± 4.0d	31.1 ± 0.7d	7	15.50	7.00
Canarium ovatum nut	29.6 ± 0.8	191 ± 4g	144 ± 5d	82.0 ± 5.0	31.1 ± 0.7d	7	15.50	5.00
Canarium ovatum pulp	43.0 ± 6.3	519 ± 6g	347 ± 4a	316.4 ± 10.7c	80.1 ± 1.6d	6	12.50	5.50
Rubus rosifolius	84.0 ± 0.6	475 ± 5h	231 ± 4f	512.5 ± 4.0b	76.2 ± 0.8d	3	1.70	3.00

Table 2 shows the radical Scavenging Values of different fruit samples of Recuenco et al., (2022). Based on the results, Garcinia binucao has the highest total phenolics with 758 mg GAE. Garcinia binucao also contained a total of flavonoids of 312 mg CE. This test results only shows that the Garcinia binucao fruit contain compounds such as phenolics and flavonoids that are commonly used as antibacterial agents.

Table 3 Agar Disk Diffusion Results

Sample/Control	Replicate 1	Replicate 2	Replicate 3	Mean ZOI (mm)	Reactivity	Inhibitory Activity
Batuan (*Garcinia binucao*) Fruit Crude Extract 50%	19.17	18.85	14.77	17.60	3	+++
Batuan (*Garcinia binucao*) Fruit Crude Extract 75%	21.53	20.79	22.93	21.75	4	+++
Batuan (*Garcinia binucao*) Fruit Crude Extract 100%	28.78	29.86	31.09	29.91	4	+++
Oxacillin (1 µg)	20.46	22.44	22.21	21.70	4	+++

Table 3 shows the antibacterial activity of Batuan (*Garcinia binucao*) fruit crude extract at 50%, 75%, and 100% concentrations compared with the control (oxacillin) against *Staphylococcus aureus*. At 50%, the extract produced inhibition zones of 19.17 mm, 18.85 mm, and 14.77 mm, with a mean of 17.60 mm, showing complete inhibitory activity (+++) and moderate reactivity (3). The 75% extract yielded inhibition zones ranging from 20.79 mm to 22.93 mm, with a mean of 21.75 mm, corresponding to complete inhibitory activity (+++) and severe reactivity (4). At 100%, the extract exhibited the strongest activity, with inhibition zones between 28.78 mm and 31.09 mm and a mean of 29.91 mm, also rated as complete (+++) with severe reactivity (4). The positive control, oxacillin, produced inhibition zones of 20.46 mm, 22.44 mm, and 22.21 mm, with a mean of 21.70 mm, likewise showing complete inhibitory activity (+++) and severe reactivity (4).

The 50% extract exhibited a reactivity rating of moderate and produced complete inhibitory activity (+++) with a mean inhibition zone of 17.60 mm. Meanwhile, the 75% and 100% concentrations and Oxacillin, all showed a reactivity rating of severe antibacterial activity, and produced complete inhibitory activity (+++) with mean inhibition zones of 21.75 mm, 29.91 mm, and

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21.70 mm, respectively. These results indicate that all concentrations of the Batuan extract were effective in inhibiting the growth of *Staphylococcus aureus*, higher concentrations demonstrated stronger antibacterial effects, indicating a concentration-dependent relationship.

Among the three concentrations, the 100% concentration of the extract showed the highest inhibitory effect, with inhibition zones measuring 28.78 mm, 29.86 mm, and 31.09 mm, yielding a mean ZOI of 29.91 mm. Which is greater than the 50% with a mean inhibition zone of 17.60 mm and 75% concentration with 21.75 mm.

Table 4 ANOVA Results for Zone of Inhibition of Batuan Extract and Control

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	239.7527	3	79.91756	32.92899	0.005788	4.066181
Within Groups	19.41573	8	2.426967	-	-	-
Total	259.1684	11	-	-	-	-

Table 4 presents the computed ANOVA results comparing the three concentrations of the Batuan fruit crude extract and the control group in terms of zone of Inhibition.

The variation between the groups produced a sum of squares (SS) of 239.75 with 3 degrees of freedom and a mean square (MS) of 79.92. In contrast, the variation within the groups had an SS of 19.42 with 8 degrees of freedom and an MS of 2.43. The calculated F-value was 32.93, with a p-value of 7.52E-05. The F-critical value was 4.07.

This implicates that the different concentrations of the Batuan fruit crude extract exhibit varying levels of effectiveness in eradicating the *Staphylococcus aureus*, and the concentration specifically the 100% of the fruit crude extract demonstrates a significantly greater effect compared to the control.

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CHAPTER FOUR
CONCLUSIONS

Different concentration of Batuan (*Garcinia binucao*) fruit crude extract were evaluated for its antibacterial activity against *Staphylococcus aureus*. Based from the findings of the study the following conclusions were drawn:

The study demonstrated that the Batuan fruit crude extract possessed antibacterial properties against *Staphylococcus aureus*. The antibacterial effect was found to be concentration-dependent. The 50% concentration of the crude extract showed moderate antibacterial activity, as evidenced by a moderate zone of inhibition in the Agar disk diffusion method. The 75% and 100% concentration of the fruit crude extract showed high antibacterial activity as evidenced by its high zone of inhibition in the Agar disk diffusion method.

The 100% concentration of Batuan fruit crude extract showed the highest antibacterial activity, presenting the largest zone of inhibition in the Agar disk diffusion assay with a mean inhibition zone of 29.91 mm. Based on the result of the One-way ANOVA, there is a statistically significant difference among the treatments and the control group given that the computed F-value (32.93) is much higher than the F-critical value (4.07), and the P-value (0.005788) is significantly less than 0.05.

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RECOMMENDATIONS

Based from the findings from this study, it is important to consider further actions to strengthen its scientific relevance. Such as testing the Batuan fruit crude extract on a much more complex biological strains from other microorganisms that causes skin infections and pneumonia. Determining the capabilities of the extract would greatly enhance its antibacterial potential.

A study has shown that MSSA's (Methicillin-resistant *Staphylococcus aureus*) resistance to antibiotics is part of a larger issue that greatly concerns healthcare and public health professionals (Bustamante et al., 2024). With this, testing the Batuan fruit crude extract against these strains will help establish its broader antibacterial potential. Another action that is to be considered is the investigation of combined effects of Batuan fruit crude extract with other conventional antibiotics. Exploring the potential of the extract with other antibiotics will greatly enhance and sustain its antibacterial activity against more complex microorganism, particularly those who have a much more resistance towards antibiotics.

A study has shown that combinations of natural compounds with conventional antibiotics enhance efficacy against resistant microorganisms by acting synergistically, modulating resistance mechanisms, or increasing drug penetration. Such as extracts combined with antibiotics like *nalidixic acid, meropenem,* or *colistin* have shown improved activity against *Salmonella*, *carbapenem*-resistant bacteria, and *colistin*-resistant bacteria, respectively. The potential of natural products is further explored through systematic reviews and the isolation of bioactive compounds like quercetin, a-mangostin, and sanguinarine which have shown promise in overcoming antibiotic resistance (Vaou et al., 2021). The extract's antibacterial effectiveness against more complex microorganisms, especially those with more antibiotic resistance, will be significantly increased and maintained by investigating its potential in combination with other antibiotics.

An action that is also to be considered is the conduct cytotoxicity assays on human cell lines. This is to determine whether this extract is harmful to therapeutic treatments. A study has shown the critical role of cytotoxicity assays in alternative medicine for assessing the safety and effectiveness of herbal extracts, essential oils, and natural products by evaluating their impact on cell viability and proliferation (Gavanji et al., 2023). These assays are crucial for identifying potentially harmful compounds, understanding the mechanism of action, and ensuring that substances intended for therapeutic use do not cause significant damage to normal cells.

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Above all, the researchers give their utmost thanks to Almighty God, who served as their source of wisdom, knowledge, and strength. His divine guidance and protection sustained them throughout the course of this endeavor and gave them hope and perseverance despite the challenges encountered.

The researchers are deeply thankful to their beloved parents for their unconditional support, both financially and emotionally. Their encouragement, sacrifices, and steadfast faith in the researchers' abilities provided the motivation needed to accomplish this study.

Special recognition is due to Teacher Edmark M. Balce, their adviser and Practical Research teacher, for his invaluable guidance, patience, and commitment in imparting knowledge. His mentorship and encouragement directed the researchers toward the proper path of academic inquiry. Additionally, to Teacher Joan Eleazar as well, for helping us with our study, both their guidance is what truly made our study to work with or without any failure.

The researchers also express their sincere appreciation to Ms. Vidalyn Faith Ramirez Eco, their Medical Technologist, for her assistance in providing essential information, access to research facilities, and valuable insights that greatly contributed to the progress of this study.

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Gratitude is also extended to Ma'am Delma Jean V. Abad, PhD, their language editor, for her careful review and refinement of the manuscript, ensuring clarity and accuracy in its presentation.

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The researchers are also grateful to Green Matter Botanical for their technical assistance in the extraction and dilution of plant samples, as well as to DOST-ITDI-STD for providing the necessary support and resources that allowed the assay testing to be conducted successfully.

We would like to express our sincere thanks to our teachers who guided and supported us during our research. To Teacher Stephanie Jane G. Nuñez, Eimeren Basto, and Joan Eleazar for their help and encouragement in developing and improving our 3D model.

To all individuals, families, and institutions who extended their guidance, support, and encouragement, the researchers express their deepest and most sincere gratitude. This study stands as a testament to your generosity and collaboration.

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APPENDICES

APPENDIX A: DOCUMENTATION

Fig 4 Gathering of Batuan Fruit

Fig 5 Authentication of Plant Material

Fig 6 Extraction of Plant Material Sent by Green Matter Botanicals

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Fig 7 Received Diluted Extracts

Fig 8 Agar Disk Diffusion Test Sent by DOST-ITDI

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APPENDIX B: LETTERS AND CERTIFICATIONS

Republic of the Philippines
Department of Education
REGION V – BICOL
SCHOOLS DIVISION OFFICE OF CAMARINES NORTE

LABO SCIENCE AND TECHNOLOGY HIGH SCHOOL
Dalas, Labo

August 4, 2025

ENGR. LEOPOLDO P. BADIOLA
PENR Officer

For:
GIL B. BADAGUAS, JR.
EMS I

Sir,

Greetings!

We are formally requesting for the certificate of plant authentication conducted by your facility on May 19, 2025, at the Provincial Environment and Natural Resources Office - Provincial Government for our research study titled **"In Vitro Evaluation of Batuan (Garcinia binucao) Crude Extract against Staphylococcus aureus"**. This study aims to determine the effectiveness and evaluate the antimicrobial activity of terpenes from Batuan (Garcinia binucao) Crude Extract against Staphylococcus aureus.

We are looking forward for your affirmative response regarding this matter.

Very truly yours,

MIKAELA N. OBUSAN
Research Leader

Noted:

EDMARK M. BALCE
Research Adviser

LAARNI N. SAMAN
Officer-in-Charge

Fig 9 Letter of Request for Certification of Plant Authentication from Provincial Environmental and Natural Resources Office (PENRO)

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Republic of the Philippines
PROVINCE OF CAMARINES NORTE
PROVINCIAL ENVIRONMENT AND NATURAL RESOURCES OFFICE

CERTIFICATION

This is to certify that the plant sample provided by the Grade 11 - Fortitude pupils of Labo Science and Technology High School is **Batuan** (*Garcinia binucao*).

This certification is issued upon the request of Ms. Mikaela N. Obusan - Team Leader of the research study entitled *"In Vitro Evaluation of Batuan (Garcinia Binucao) Crude Extract Against Streptococcus Pneumoniae"*.

Issued this 21st day of May, 2025 at Daet, Camarines Norte.

GIL B. BADAGUAS, JR., RPF
Environmental Mngt. Specialist II
Licensed Forester-PRC ID No. 10303

Fig 10 Certification of Plant Authentication from Provincial Environmental and Natural Resources Office (PENRO)

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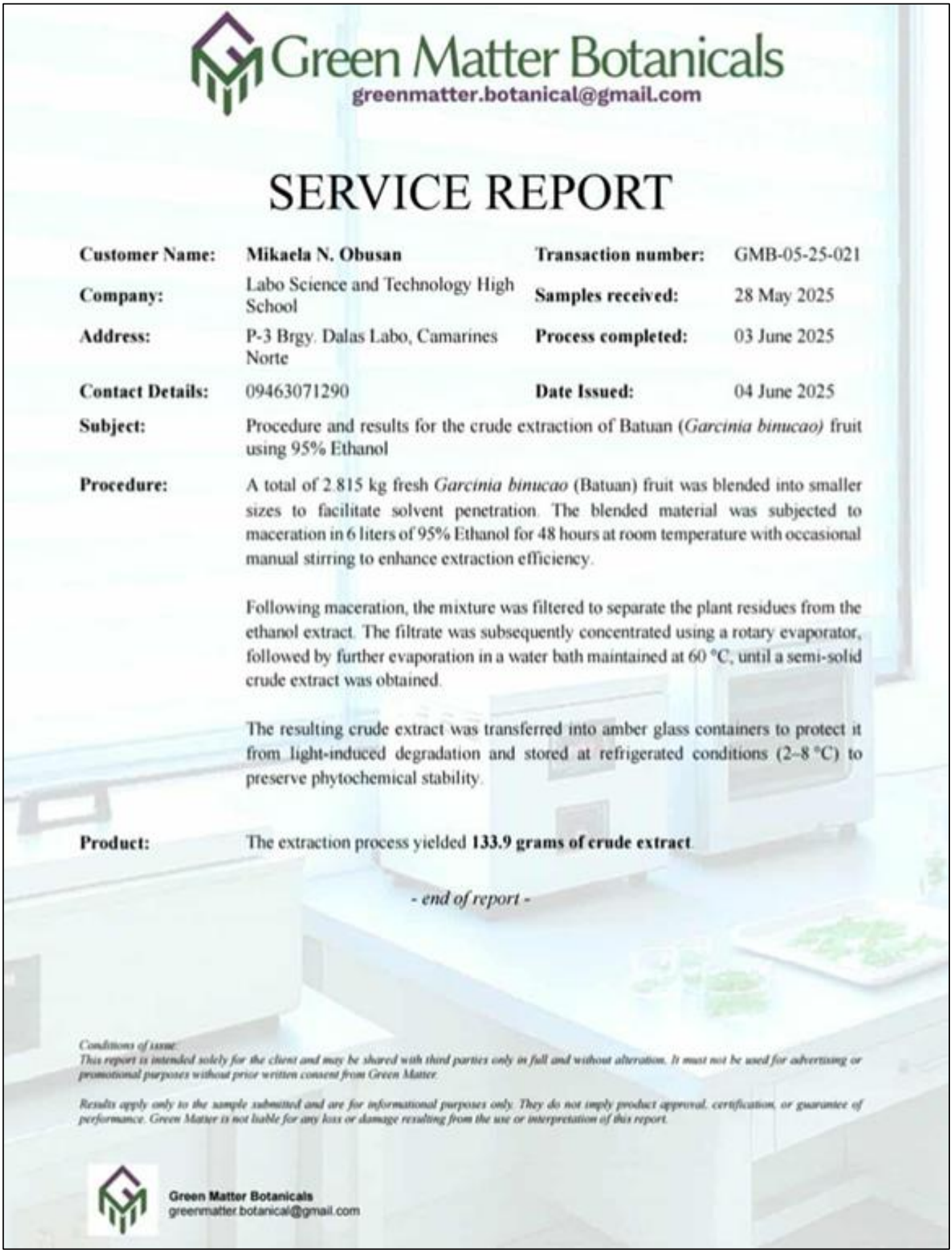

Green Matter Botanicals
greenmatter.botanical@gmail.com

SERVICE REPORT

Customer Name:	**Mikaela N. Obusan**	**Transaction number:**	GMB-05-25-021
Company:	Labo Science and Technology High School	**Samples received:**	28 May 2025
Address:	P-3 Brgy. Dalas Labo, Camarines Norte	**Process completed:**	03 June 2025
Contact Details:	09463071290	**Date Issued:**	04 June 2025

Subject: Procedure and results for the crude extraction of Batuan (*Garcinia binucao)* fruit using 95% Ethanol

Procedure: A total of 2.815 kg fresh *Garcinia binucao* (Batuan) fruit was blended into smaller sizes to facilitate solvent penetration. The blended material was subjected to maceration in 6 liters of 95% Ethanol for 48 hours at room temperature with occasional manual stirring to enhance extraction efficiency.

Following maceration, the mixture was filtered to separate the plant residues from the ethanol extract. The filtrate was subsequently concentrated using a rotary evaporator, followed by further evaporation in a water bath maintained at 60 °C, until a semi-solid crude extract was obtained.

The resulting crude extract was transferred into amber glass containers to protect it from light-induced degradation and stored at refrigerated conditions (2–8 °C) to preserve phytochemical stability.

Product: The extraction process yielded **133.9 grams of crude extract**.

- end of report -

Conditions of use:
This report is intended solely for the client and may be shared with third parties only in full and without alteration. It must not be used for advertising or promotional purposes without prior written consent from Green Matter.

Results apply only to the sample submitted and are for informational purposes only. They do not imply product approval, certification, or guarantee of performance. Green Matter is not liable for any loss or damage resulting from the use or interpretation of this report.

Green Matter Botanicals
greenmatter.botanical@gmail.com

Fig 11 Crude Extraction Service Report from Green Matter Botanicals

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ANTIMICROBIAL ASSAYS

A. Preparation of Test Organisms

1. Inoculate bacterial isolate to 10-ml Tryptic Soy Broth (TSB) and incubate overnight at 35°C.
2. Use overnight culture for Step B.

B. Preparation of Antimicrobial Assay Plates

1. Adjust the overnight culture to a turbidity comparable to 0.5 McFarland Standard.
2. Add 200uL of the adjusted bacterial suspension to a standard-size sterile petri dish/plate.
3. Add approximately 15ml-20ml of Mueller-Hinton Agar (prepared as per manufacturer's recommendations), swirl, and allow plates to congeal and dry.
4. Incubate plates at 35°C (20 minutes for *E. coli*, 1 hour for other bacteria) before adding the filter paper discs with samples (see Step C).

C. Addition of samples to filter paper discs (10-mm)

C1. For **non-viscous and less viscous extracts/liquids.** Pipette 10uL of the sample into the 10-mm sterile filter paper discs, then add the discs into the assay plates prepared from Step B.

C2. For **viscous extracts/liquids, creams, or ointments.** Dip filter paper discs in the extract, and allow the extract to spread into the paper. Remove excess extracts by tapping into the sides of the container, then add the discs into the assay plates prepared from Step B.

C3. For **dry extracts and solid samples**. Take a sufficient amount of the extract/sample and add 1mL of water (or depending on the agreed upon concentration, if any) to dissolve. Filter paper discs will then be dipped into the sample in the manner described in C2.

D. Use of positive controls

Commercially available antibiotic discs are used as positive controls. Should the customer require to use another set of positive controls, the addition of positive control to the plate will be similar to the procedure on Step C (depending on the form of the positive control provided), unless the customer has another procedure on the preparation of positive control. This is upon approval of the laboratory.

E. Incubation and Interpretation of Results

1. Incubate the plates inverted overnight at 35°C.
2. Observe for zones of inhibition after incubation. Measure the zones of inhibition using caliper. If there are no zones surrounding the paper discs, aseptically lift the paper and observe the area under the sample. Report results as follows:

Reactivity Rating: 0 – None (No detectable zone around or under specimen)
1 – Slight (Some malformed or degenerated cells under the specimen)
2 – Mild (zone limited under the specimen)
3 – Moderate (zone extends 5 to 10 mm beyond specimen)
4 – Severe (zone extends greater than 10 mm beyond specimen)

Inhibitory Activity Rating: (+++) complete; (++) partial; (+) slight, and (-) negative

Reference: USP 30 – NF 25, 2007, <Biological Reactivity Test, In vitro

Fig 12 Agar Disk Diffusion Service Report from Department of Science and Technology–Industrial Technology Development Institute (DOST-ITDI)

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REPUBLIC OF THE PHILIPPINES
DEPARTMENT OF SCIENCE AND TECHNOLOGY
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
STANDARDS AND TESTING DIVISION

A Member of DOST OneLab

Sample/ Control	*Staphylococcus aureus (ATCC 6538)*					
	Replicate 1	Replicate 2	Replicate 3	Total Mean Zone of Inhibition (mm)	Reactivity	Inhibitory Activity
Batuan (Garcinia binucao) Crude Extract 50% (10 mm)	19.17	18.85	14.77	17.60	3	+++
Batuan (Garcinia binucao) Crude Extract 75% (10 mm)	21.53	20.79	22.93	21.75	4	+++
Batuan (Garcinia binucao) Crude Extract 100% (10 mm)	28.78	29.86	31.09	29.91	4	+++
Positive Control: *Oxacillin 1 ug* (6 mm)	20.46	22.44	22.21	21.70	4	+++
Negative Control: Sample-free disc (10 mm)	0.00	0.00	0.00	0.00	0	(-)

Reactivity Rating: 0 – None (No detectable zone around or under specimen)
1 – Slight (Some malformed or degenerated cells under the specimen)
2 – Mild (zone limited under the specimen)
3 – Moderate (zone extends 5 to 10 mm beyond specimen)
4 – Severe (zone extends greater than 10 mm beyond specimen)

Inhibitory Activity Rating: (+++) complete; (++) partial; (+) slight, and (-) negative

Test Reference: United States Pharmacopoeia 30-NF 25, 2007 <87> Biological Reactivity Tests, In vitro
Test Method: Disc Diffusion Method

VALIDITY OF THE TEST REPORT: The test results are those obtained at the time of the test and pertain only to the sample(s) received by the Laboratory of this Institute. *Codes and words in Italics are provided by the customer and quoted solely for the customer's reference; significance of these codes and words is not verified by the Laboratory.* This report is not to be used for advertising purposes or sales promotion. This report shall not be reproduced except in full without the approval of the Standards and Testing Division.

GP 5.10-01-F06

AGNES P. DE ASIS, RMT, MSc
Head, Microbiology Section

Issued under the Authority of:

MARLON SA AGUINALDO, RMT
Officer-in-Charge
Standards and Testing Division

ITDI-072025-MIC-0268
Date of Issue: August 05, 2025
Page 2 of 2

Quality Life and Products through Testing

STD Building, DOST Complex, Gen. Santos Ave., Bicutan, Taguig City, Metro Manila 1631, Philippines
Tel Nos.: (+632) 8-683-7750 to 69 locals 2188 (Receiving and Releasing Unit) / 2198 (Division Chief);
Email Address: std@itdi.dost.gov.ph; Website: http://itdi.dost.gov.ph;
Social Media Account: https://www.facebook.com/std.itdi.dost/

Fig 13 Agar Disk Diffusion Assay Results

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STATISTICIAN'S CERTIFICATION

This document certifies that the statistical treatment and analysis of the data in the research study entitled:

Antibacterial Potential of Batuan (*Garcinia binucao*) Fruit Crude Extract Against *Staphylococcus aureus*: An In Vitro Study Employing Agar Disc Diffusion Assay

Authored by:

Faith Erika C. Flores
Kimberly J. Alarma
Marc Paul L. Perillo

of Senior High Department and Research Department
Labo Science and Technology High School
have been reviewed and validated by the undersigned statistician.

Issued on September 27, 2025
Issued by:

NIEVES V. GUARDIAN
Statistician

BS in Civil Engineering 2015 (Camarines Norte State College)

Fig 14 Statistician's Certification

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Republic of the Philippines
Department of Education
Region V-Bicol
SCHOOLS DIVISION OFFICE CAMARINES NORTE
LABO SCIENCE AND TECHNOLOGY HIGH SCHOOL
Dalas, Labo, Camarines Norte

CERTIFICATION OF SCIENTIFIC REVIEW COMMITTEE

This is to certify that the study titled "In Vitro Evaluation of Batuan (*Garcinia binucao*) Fruit Crude Extract Against *Staphylococcus aureus*" by the following researchers; Faith Erika C. Flores, Kimberly J. Alarma and Marc Paul L. Perillo was reviewed by the school Scientific Review Committee on September 29, 2025.

Given this 29th day of September, 2025 at Labo Science and Technology High School, Dalas, Labo, Camarines Norte.

Scientific Review Committee (SRC)

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Chairman

ENIRISA B. PALIVINO
Vice Chairman

EIMEREN E. BASTO
Ethics Reviewer

JACQUELINE V. CERENO
Plagiarism Checker

DR. JOHN JEFFERSON A. VELASCO
Bibliographic Tracer

CECILE F. BUSTAMANTE
Technical Expert

Fig 15 Certification of Scientific Review Committee

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CERTIFICATION OF THE SECRETARY

This is to certify that the suggestions given by the Panel of Examiners in connection with the Oral Examination of Practical Research in the defense of this thesis titled, **Antibacterial Potential of Batuan (*Garcinia binucao*) Fruit Crude Extract Against *Staphylococcus aureus*: An In Vitro Study Employing Agar Disc Diffusion Assay**, were complied with.

KIMBERLY J. ALARMA
Secretary

CERTIFICATION OF THE LANGUAGE EDITOR

This is to certify that the thesis of **Faith Erika C. Flores, Mikaela N. Obusan, Kimberly J. Alarma and Marc Paul L. Perillo** titled, **Antibacterial Potential of Batuan (*Garcinia binucao*) Fruit Crude Extract Against *Staphylococcus aureus*: An In Vitro Study Employing Agar Disc Diffusion Assay**, was edited by the undersigned.

DELMA JEAN V. ABAD, PhD
Language Editor

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APPENDIX C: LABORATORY RESULTS AND STATISTICAL COMPUTATION

Table 5 Laboratory Result

Sample/Control	***Staphylococcus aureus* (ATCC 6538)**					
	Replicate 1	**Replicate 2**	**Replicate 3**	**Mean ZOI (mm)**	**Reactivity**	**Inhibitory Activity**
Batuan (*Garcinia binucao*) Fruit Crude Extract 50%	19.17	18.85	14.77	17.60	3	+++
Batuan (*Garcinia binucao*) Fruit Crude Extract 75%	21.53	20.79	22.93	21.75	4	+++
Batuan (*Garcinia binucao*) Fruit Crude Extract 100%	28.78	29.86	31.09	29.91	4	+++
Oxacillin (1 µg)	20.46	22.44	22.21	21.70	4	+++

Table 6 Statistical Computation

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	239.7527	3	79.91756	32.92899	0.005788	4.066181
Within Groups	19.41573	8	2.426967			
Total	259.1684	11				

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APPENDIX D: MATERIALS USED AND COST ANALYSIS

Table 7 Cost Analysis

Materials	Quantity	Price
Rectangular White Plastic Crate	1 Piece	Php 35.00

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APPENDIX E: ACTUAL COMPUTATION

Table 8 Actual Computation

Materials	Quantity	Price
Transportation	N/A	Php 4000.00
Proxy (UPLB)	N/A	Php 300.00
Service Fee for plant samples (Fresh Plant Extraction - GMB)	1 kg	Php 1700.00
Service Fee for additional plant samples (Fresh Plant Extraction – GMB)	2 kg	Php 1800.00
95% Ethanol	10 L	Php 1080.00
Amber Bottles	2 pieces	Php 80.00
Dilution of Extracts	N/A	Php 200.00
Proxy (DOST-ITDI-STD)	N/A	Php 1,100.00
Agar Disk Diffusion Assays	3 Tests	Php 1,800.00