



ASSESSMENT OF THE INHIBITORY POTENTIAL OF *Capsicum frutescens* (Siling Labuyo) LEAVES EXTRACT AGAINST *Acinetobacter baumannii*

Marjorie Anne Dimaano 1st, Dr. Ronalyn Sanchez, RMT, MSc 2nd

University of Perpetual Help - System Laguna, Biñan, Laguna, Philippines

Abstract: This study investigated the antibacterial potential of *Capsicum frutescens* (siling labuyo) leaf extracts against the multidrug-resistant bacterium *Acinetobacter baumannii*, a common cause of hospital-acquired infections. Researchers tested four concentrations (25%, 50%, 75%, and 100%) of the leaf extract using the disc diffusion method. The zones of inhibition were measured and compared to the antibiotic Imipenem. The results indicated a concentration-dependent antibacterial effect, with the 100% extract showing the largest inhibition zone (13.33 mm). Statistical analysis confirmed significant differences in activity among the concentrations. However, even the highest concentration's effect was considerably less than that of Imipenem (25 mm). The study concluded that while *C. frutescens* leaf extracts exhibit moderate antibacterial activity against *A. baumannii* at high concentrations, they are not a viable alternative to current antibiotics. Further research focusing on refinement and isolation of active compounds is suggested to potentially enhance its therapeutic potential.

I. INTRODUCTION

Hospital-acquired infections (HAIs) are a highly public health priority worldwide, contributing to significantly increasing morbidity and mortality, and healthcare cost. These infections typically originate within clinical environments—such as hospitals, nursing homes, and other healthcare facilities—where immunocompromised patients are at increased risk of exposure to opportunistic pathogens (Umemura et al., 2022). Proudly one of the most common HAIs, *Acinetobacter baumannii* can survive on inanimate hosts and exhibit some resistance to various toxic conditions. Over the past decade, *Acinetobacter baumannii* has gained increased attention due to a broad spectrum of antibiotic resistance which prevents many conventional treatments from working (Tiku et al., 2021).

The escalating antibiotic resistance in *Acinetobacter baumannii* poses a significant threat to global health. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains are increasingly tied to severe clinical manifestations—particularly in intensive care units (ICUs)—including pneumonia, bloodstream infections, and wound infections (Tiku et al., 2021). International health organizations have underscored the urgent need for novel or alternative treatment strategies (Nwobodo et al., 2022), warning that a failure to discover effective interventions may result in prolonged hospital stays, greater healthcare costs, and increased mortality. These outcomes underscore the dire implications of uncontrolled antibiotic resistance. At the same time, the ongoing resistance crisis has stimulated significant scientific interest in plant-derived substances as potential antimicrobial agents.

Traditionally, medicinal plants have been employed for centuries across diverse cultures to manage myriad health conditions (Stan et al., 2021). Their therapeutic properties are frequently attributed to bioactive phytochemicals—such as alkaloids, phenolic acids, flavonoids, and capsaicinoids—that disrupt bacterial cell membranes, inhibit key enzymes, or interfere with metabolic pathways (Hassan et al., 2021). Of these plants,

Capsicum frutescens (Siling labuyo), has gotten considerable attention due to its well-established antimicrobial activities and capsaicinoid fruit extracts (Hassan et al., 2021).

However, the inhibitory efficacy of *Capsicum frutescens* leaf extracts are still not well established. Many preliminary data suggest that leaves may contain phenolic compounds, flavonoids, or other secondary metabolites that inhibit or kill the bacteria (Hassan et al., 2021). Most research to date has focused on the fruit of *Capsicum frutescens*, possibly overlooking the leaf's distinct phytochemical profile. Furthermore, although some studies have hinted at the leaves' potential against common bacterial pathogens, conclusive data on their specific influence against multidrug-resistant bacteria—particularly *Acinetobacter baumannii*—are insufficient. Critical questions remain regarding optimal extraction methods, effective concentrations, and the precise mechanism by which leaf extracts inhibit or kill *Acinetobacter baumannii*. Addressing these questions is essential for broadening our antimicrobial arsenal, especially given the growing threat of antibiotic resistance.

Thus, to address the problem, the present study aims to assess the inhibitory potential of *Capsicum frutescens* leaf extracts against *Acinetobacter baumannii* under controlled laboratory conditions. By evaluating four distinct concentrations—25%, 50%, 75%, and 100%—the research examines whether these varying levels of leaf extracts can produce statistically significant inhibitory effects on bacterial growth.

Theoretical/Conceptual Framework

This study was anchored in two major theories that collectively explain how microbial pathogens cause disease and how plant-derived substances can inhibit these pathogens. First, the Germ Theory of Disease posits that many illnesses result from the invasion and proliferation of microorganisms—such as bacteria, viruses, or

Fungi in a host (Llorens & Agustí-Brisach, 2022). In the context of this research, *Acinetobacter baumannii* is recognized as a significant agent in hospital-acquired infections; thus, isolating and studying *Acinetobacter baumannii* under controlled conditions leverages the Germ Theory's principle that effectively identifying and inhibiting the bacterial agent can mitigate or reduce infection rates.

Second, the Phytochemical Antimicrobial Theory holds that bioactive compounds derived from plants, including those found in *Capsicum frutescens* leaves, can inhibit bacterial growth by disrupting cell membranes, inactivating key enzymes, or interfering with essential metabolic processes (Hassan et al., 2021; Huang et al., 2021). These phytochemicals—such as capsaicinoids, phenolic acids, and flavonoids—are hypothesized to exert bactericidal or bacteriostatic effects on target pathogens, including *Acinetobacter baumannii* (Periferakis et al., 2023).

By integrating these two theoretical perspectives, the study underscored both the critical role of microbial pathogens in causing disease (Germ Theory) and the potential for plant-derived agents to act as viable inhibitors (Phytochemical Antimicrobial Theory). Systematic experimentation was thus employed to examine whether increasing concentrations of *Capsicum frutescens* leaf extracts can effectively inhibit the growth of *Acinetobacter baumannii*, offering an alternative or complementary approach for managing antibiotic-resistant infections.

Operational Framework

This study applied the Input-Process-Output (IPO) model to evaluate the inhibitory potential of *Capsicum frutescens* leaf extracts against *Acinetobacter baumannii*. As inputs, the researcher prepared both the dried and extracted leaves of *Capsicum frutescens* (collected and processed through an Ethanol solvent extraction) and the standardized *Acinetobacter baumannii* cultures. The process encompassed leaf authentication, solvent extraction, and in vitro assays (disc or well diffusion) to measure inhibition zones, followed by statistical evaluations—such as One-Way ANOVA—to determine significant differences in inhibition among the four extract concentrations (25%, 50%, 75%, and 100%). In this context, the independent variable (IV) is the varying *Capsicum frutescens* leaf extract concentrations, while the dependent variable (DV) is the inhibitory potential against *Acinetobacter baumannii*, measured through zones of inhibition or colony count reduction. Finally, the output consisted of quantitative data identifying which concentration yields the most effective inhibition and included conclusions and recommendations for future research into plant-based strategies against antibiotic-resistant pathogens, thereby guiding practical applications of the study's findings.

MODELING AND ANALYSIS

Operational Model

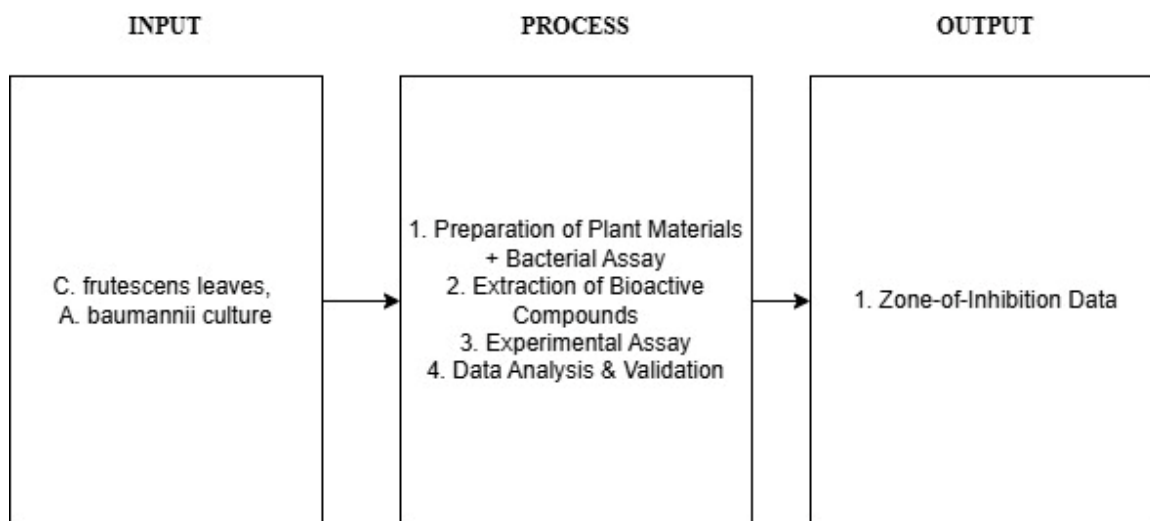


Figure 1. Operational Model Using the Input-Process-Output (IPO) Framework

II. RESEARCH METHODOLOGY

The methodological approach employed to assess the antibacterial potential of *Capsicum frutescens* leaf extracts against *Acinetobacter baumannii*, outlining the research design, Source of data, preparation of materials, assay procedures, and statistical analyses conducted.

Research Design

This study utilized an experimental research design to evaluate the inhibitory potential of *Capsicum frutescens* leaf extracts against *Acinetobacter baumannii*. According to Zubair (2022), an experimental design allows the researcher to manipulate one or more variables (in this case, extract concentrations) and measure the resulting changes in a dependent variable (bacterial growth inhibition). This approach is considered rigorous because it can establish cause-and-effect relationships under controlled conditions.

A posttest-only control group format was adopted, consistent with the guidelines of Blakstad (2018) for ensuring valid and reliable experimental outcomes. Under this design, varying extract concentrations (25%, 50%, 75%, and 100%) served as treatment groups, while a positive control (Imipenem) was used to compare the efficacy of known antimicrobials. By systematically applying these treatments to *Acinetobacter baumannii* cultures, the study could ascertain whether significant differences in inhibitory activity existed among the tested concentrations.

Sources of Data

The primary data for this study comprises the empirical results obtained from disc diffusion assays. Specifically, these include measured zones of inhibition (in millimeters) produced by four concentrations of *Capsicum frutescens* leaf extracts (25%, 50%, 75%, 100%) and an Imipenem disc (positive control) against *Acinetobacter baumannii*. Observations were recorded in triplicate under controlled laboratory conditions, yielding the quantitative outcomes that serve as the core of the statistical analyses.

Preparation of Materials

Tools and Equipment

The list of the key tools and instruments used by the researcher are Water bath, Rotary evaporator, Micropipettes, Biosafety Cabinet Level II, Autoclave, Analytical balance, pH meter or pH strips, Digital caliper, Flask, Autoclavable bottles and Petri dishes. The tools and instruments listed above play crucial roles in the study's methodology, ensuring accuracy, sterility, and the validity of results.

Extraction and Preparation: A water bath or rotary evaporator was used to concentrate the extract by removing the solvent. This step is essential to isolate the active compounds from the plant material. Micropipettes were used for precise measurement of solvents and extracts, which is critical for creating accurate concentrations and dosages.

Sterile Technique and Safety: A Biosafety Cabinet Level II provided a sterile environment for handling bacterial cultures, minimizing contamination and protecting the researcher. The autoclave was used to sterilize media, glassware (flasks, autoclavable bottles), and other equipment (petri dishes, forceps) to prevent unwanted microbial growth.

Measurement and Analysis: An analytical balance with a calibration certificate was used for accurate weighing of materials, crucial for preparing solutions and extracts. A pH meter or pH strips were used to check the acidity or alkalinity of solutions, which can affect the activity of the extracts. A digital caliper was used to measure the zones of inhibition in the disc diffusion assay, providing quantitative data on the antibacterial activity of the extracts.

Other Equipment: Flasks were used to hold and mix solutions. Autoclavable bottles were used to sterilize and store the agar solution. Petri dishes were used as containers for the agar plates in the disc diffusion assay.

These tools collectively enabled the researchers to conduct a controlled experiment, from preparing the plant extracts to analyzing their effects on bacterial growth.

Collection, Authentication, and Preparation of Plant Materials

1.5 kg of fresh *Capsicum frutescens* (siling labuyo) leaves were sourced from a verified local supplier. To confirm the taxonomic identity, the Bureau of Plant Industry examined and certified the plant materials belonging to *Capsicum frutescens*. Subsequently, from March 11–19, 2025, the Department of Science and Technology (DOST–Region 4A, Chemicals and Energy Division) dried and processed the leaves by official protocols (see Figure 2). Specifically, 395 g of dried leaf material was macerated in 3 L of 95% ethyl alcohol for 48 hours, with occasional stirring to ensure thorough solvent contact. The mixture was then filtered, and the resulting filtrate concentrated in a water bath set at 60°C to produce a semi-solid extract. This semi-solid extract was stored at 2–8°C until further use.

Procurement of Test Organism

Pure *Acinetobacter baumannii* cultures were acquired from the De La Salle Medical and Health Sciences Institute (Governor D. Mangubat St., Avenue, Dasmariñas, Cavite), accompanied by official documentation attesting to both purity and antibiotic resistance profiles. To confirm viability and reduce the likelihood of contamination, the strain was sub-cultured onto MacConkey agar. Thereafter, colonies were standardized to a 0.5 McFarland turbidity level—equivalent to roughly 1.5×10^8 CFU/mL—before inoculating Mueller-Hinton agar plates for the disc diffusion assay. This process aligns with recommended clinical laboratory protocols aimed at ensuring a uniform bacterial load across experimental trials. The organism, recognized for its multidrug-resistant (MDR) traits, was stored and maintained under prescribed conditions at Westlake Medical Center Laboratory to preserve its purity and virulence for the duration of the study.

Mueller Hinton Agar Preparation

The requisite amount of dehydrated MHA powder was measured according to the manufacturer's guidelines, 38 g per liter of distilled water, then gradually introduced into a flask containing the calculated volume of distilled water. Throughout this process, the mixture was stirred continuously to prevent clumping. Next, the suspension was gently heated, allowing the powder to dissolve completely without scorching. Once fully dissolved, the agar solution was transferred to autoclavable bottles and sterilized via autoclave at 121°C and 15 psi for 15 minutes.

After sterilization, the bottles were cooled to approximately 45–50°C before the molten agar was aseptically dispensed into sterile Petri dishes—typically at a depth of 4–5 mm—to form uniform media layers. Plates were left undisturbed until the agar solidified; a subset of uninoculated plates was then incubated at 35–37°C for 24 hours to confirm sterility. Absence of microbial growth in these control plates indicated that the prepared MHA was free from contamination and suitable for subsequent disc diffusion assays.

Instrumentation

In this study, leaf extracts from *Capsicum frutescens* were prepared to assess their inhibitory potential against *Acinetobacter baumannii*. The leaves were dried, pulverized, and extracted using the procedure verified by DOST–Region 4A. A rotary evaporator or water bath was used to remove residual solvents. Micropipettes (calibrated according to Zubair’s (2022b) guidelines) ensured accurate measurement of solvents and extracts.

To maintain a sterile work environment, all bacterial handling procedures were conducted in a Biosafety Cabinet Level II at Westlake Medical Center. Media, forceps, and petri dishes were sterilized in an autoclave (121°C, 15 psi) to prevent cross-contamination. The analytical balance had an updated certificate of calibration from the institution’s engineering department, and a pH meter or pH strips were used as needed to check solvent acidity or alkalinity.

Data Gathering Procedure

To commence the study, formal request letters were dispatched to the Department of Science and Technology (DOST–Region 4A), De La Salle Medical and Health Sciences Institute, and Westlake Medical Center, securing permission for each research phase. The Technical Service Report (Appendix X) provides further details on the official extraction procedure.

Susceptibility Testing of *Acinetobacter baumannii* against Plant Extracts

The susceptibility test in the study was conducted using the Kirby-Bauer disc diffusion method.

Preparation of Bacterial Suspension: A standardized bacterial suspension of *Acinetobacter baumannii* was prepared. The turbidity of this suspension was adjusted to match a 0.5 McFarland standard. This standardization ensures a consistent bacterial concentration for the test.

Inoculation of Agar Plates: The standardized bacterial suspension was uniformly inoculated onto Mueller-Hinton agar plates using the spread plate technique. This creates an even lawn of bacteria on the agar surface.

Application of Test Discs: Sterile 6 mm discs were impregnated with varying concentrations of the *Capsicum frutescens* leaf extract. A commercially prepared imipenem disc was used as a positive control. These discs were aseptically placed equidistantly on the agar surface.

Incubation: The inoculated plates were incubated at 37°C for 24 hours. This allows for bacterial growth and the diffusion of the test substances from the discs into the agar.

Measurement of Zones of Inhibition: After incubation, the zones of inhibition around each disc were examined. The diameter of these zones, which indicate the inhibition of bacterial growth, was measured using a digital caliper.

Interpretation of Results: The measured zones of inhibition were interpreted to determine the antibacterial activity of the extract.

Evaluation And Scoring

This study employed a rubric-based assessment to evaluate the antibacterial efficacy of *Capsicum frutescens* leaf extracts, focusing on zones of inhibition (ZOI) measured in millimeters. To ensure reliability, three experienced Medical Technologists reviewed and validated the rubric, aligning zone-of-inhibition thresholds with recognized interpretative standards.

Verbal Description	Zone-of-Inhibition Range	Interpretation
The leaf extract produced a zone of inhibition comparable to or exceeding the positive control (antibiotic).	≥ 14 mm	Susceptible
The leaf extract produced a partial inhibitory effect, evidenced by a smaller, measurable ZOI.	12–13 mm	Intermediate
No visible zone of inhibition was observed, indicating minimal or no inhibitory activity.	≤ 11 mm	Resistant

Scoring and Interpretation of Inhibitory Potential of *Capsicum frutescens* Leaf Extracts

Statistical Treatment of Data

The following statistical treatment was used to answer the research questions. First, to determine the zones of inhibition produced by each concentration (100%, 75%, 50%, 25%) of *Capsicum frutescens* leaf extracts compared to the positive control, the researchers measured the zone of inhibition in millimeter zone area. The researcher used the average in different extract concentrations and got the overall average together with the evaluation and interpretation rubric.

Second, to ascertain the significant difference in the zones of inhibition among the different treatments and the positive control, an analysis of variance (ANOVA) was employed. This also includes the comparison among their means and standard deviations. The researcher used the mean and standard deviation in comparison with the concentration (100%, 75%, 50%, 25%) of *Capsicum frutescens* leaf extract to the inhibitory effect against the *Acinetobacter baumannii*.

Ethical Consideration

All work involving *Acinetobacter baumannii* was conducted under Biosafety Level II conditions at Westlake Medical Center. Prior to each experiment, surfaces were disinfected with sodium hypochlorite or phenolic solutions to reduce contamination from unrelated agents. Throughout the experimental procedures, researchers wore protective equipment (gowns, gloves, masks, eyewear), and at the end of each run, all cultures and consumables were autoclaved at 121°C and 15 psi for at least 15 minutes to inactivate any remaining pathogens. Surfaces and equipment were then disinfected (or sterilized, if autoclave-compatible) to ensure a safe environment for subsequent work, consistent with WHO guidelines.

III. RESULTS AND DISCUSSION

The results and discussion of the antibacterial activity of *Capsicum frutescens* leaf extract against *Acinetobacter baumannii*, as determined by the Kirby-Bauer disc diffusion method.

1. Zones of Inhibition Across Different Concentrations Compared to the Positive Control

Disc diffusion tests were performed on Mueller-Hinton agar plates inoculated with a standardized *Acinetobacter baumannii* culture. Discs impregnated with the four concentrations (25%, 50%, 75%, 100%) were tested alongside an Imipenem disc (25 mm) as the positive control. Table 1 shows the three-trial measurements for each extract.

Table 1 shows the zones of inhibition produced by each of the following concentrations of (100%, 75%, 50%, 25%) *Capsicum frutescens* leaf extracts and the positive control (standard antibiotic disc). The 100% extract has an average of 13.33 raw zone inhibition measurement coming from the three trials which could mean that the leaf extract produced a partial inhibitory effect, evidenced by a smaller, measurable ZOI.

Table 1. Measurement of Zone inhibition in millimeters of different concentrations and Positive Control

Concentration	Disc 1	trial 2	Disc 3	Trial 4	Mean of Zone of Inhibition	Interpretation
100% Extract	14	13	13		13.33	Intermediate
75% Extract	12	9	6		9.00	Resistant
50% Extract	6	6	6		6.00	Resistant
25% Extract	6	6	6		6.00	Resistant
Positive Control (Imipenem)	25 mm consistently across all trials					Susceptible

The overall average zone of inhibition across all extract concentrations is 8.58 mm, which also lies in the resistant range of (≤ 11 mm). This means there is no visible zone of inhibition observed, indicating minimal or no inhibitory activity. In contrast, the positive control consistently produced a 25 mm inhibition zone, highlighting the superior potency of standard antibiotics relative to plant-based treatments. This minimal or resistant antibacterial efficacy may be attributed to several factors:

Firstly, *Acinetobacter baumannii* is a Gram-negative bacterium known for its highly impermeable outer membrane, which restricts the penetration of many antimicrobial agents, including phytochemicals, therefore this pathogen possesses a variety of intrinsic and acquired resistance mechanisms, such as efflux pumps and enzymatic degradation, which further diminish the susceptibility to natural products (Vila et al., 2017). It is also possible that *Acinetobacter baumannii* is not the most susceptible organism to the particular phytochemicals found in *Capsicum frutescens* leaves. Different bacteria, fungi, or other pathogens might respond more favorably.

Secondly, while *Capsicum frutescens* is recognized for its bioactive compounds, such as capsaicinoids and flavonoids, their concentration and bioavailability in crude leaf extracts may be insufficient to exert a strong bactericidal effect, especially in the absence of purification or synergistic enhancement. Moreover, phytochemicals can vary significantly in potency based on plant part used, extraction method, solvent type, and concentration, all of which influence their efficacy in antimicrobial assays. This finding aligns with existing literature, where natural extracts generally demonstrate lower efficacy against multidrug-resistant Gram-negative pathogens compared to conventional antibiotics (Hung et al., 2021).

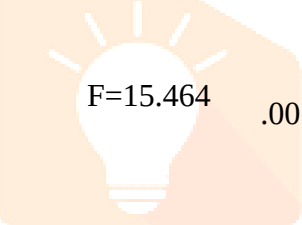
Third, other factors like choice of solvent, temperature, and duration of extraction can influence the yield and stability of phytochemicals.

2. Statistical Comparison of Inhibition Zones of *Capsicum frutescens* Extract vs. Positive Control

From Table 2 presented below, the overall ANOVA result indicates a significant difference among the four plant-based treatments ($F = 15.464$, $p = 0.001$), prompting rejection of the null hypothesis. Post-hoc Scheffé analysis further reveals that the 100% extract (mean ZOI = 13.33 mm, SD = 0.3333) achieved a significantly larger inhibition zone compared to both the 50% (6.00 mm) and 25% (6.00 mm) extracts. Meanwhile, no statistically significant difference was detected between 100% and 75% extracts, or among the lower three concentrations themselves (75%, 50%, 25%).

A positive control (Imipenem disc) consistently registered a 25 mm zone of inhibition, exceeding that of all *Capsicum frutescens* extracts. Thus, additional comparison indicates that each extract group is significantly less effective than the standard antibiotic disc. This outcome suggests that, while the 100% leaf extract exhibits the greatest inhibitory activity among the plant-based groups, it remains inferior to the positive control in terms of overall efficacy against *Acinetobacter baumannii*.

Table 2. Significant Difference in the Inhibitory Potential of *Capsicum frutescens*

Extract	Mean	Standard Deviation	Test Statistics/ Inferential Statistics	p-value	Decision	Interpretation
100% Extract	13.33	.3333	 $F=15.464$	.001*	H ₀ rejected	Significant
75% Extract	9.00	1.732				
50% Extract	6.00	.0000				
25% Extract	6.00	.0000				

*Significant@ $p\text{-value} = 0.5$

This inferior performance may be attributed to several factors: lower phytochemical concentrations in leaves compared to fruits (Moghadam et al., 2023), the inherent resistance mechanisms of *Acinetobacter baumannii*, including robust outer membranes, efflux pumps, and biofilm formation (Shahin et al., 2025; Shahryari et al., 2021), and the use of a single-solvent maceration method may compromise key bioactive compounds, aligning with research advocating more advanced extraction techniques (Ganić et al., 2022; Guo et al., 2021). Consequently, even though higher concentration fosters better inhibition, it still falls short of replicating the potent effect of a well-established antibiotic. Further research employing optimized extraction techniques and potentially different plant parts is warranted to enhance antimicrobial activity.

3. Effective Concentration of *Capsicum Frutescens* Leaf Extract Against *Acinetobacter baumannii*

While table 2 explained that One-Way ANOVA was conducted to compare the mean zones of inhibition produced by each of the four *Capsicum frutescens* extract concentrations (25%, 50%, 75%, 100%), tested in triplicate, yielding a significant result ($F = 15.464$, $p = 0.001$) that indicates at least one concentration differed substantially from the others.

Table 3. Concentration with the greatest inhibitory effect against *A. baumannii* using Scheffe post hoc test

Comparison of Different Concentrations of Extracts	Mean Difference	p-value	Interpretation
100% vs 75%	4.33	> 0.05	Not significantly different
100% vs 50%	7.33	< 0.05	Significantly different
100% vs 25%	7.33	< 0.05	Significantly different
75% vs 50%	3.00	> 0.05	Not significantly different
75% vs 25%	3.00	> 0.05	Not significantly different
50% vs 25%	0.00	> 0.05	Not significantly different

***Significant@ p-value = 0.5**

Table 3 above shows that Scheffé post-hoc test clarified that the 100% extract (mean zone of 13.33 mm, SD = 0.3333) significantly outperformed the 50% and 25% extracts (both 6.00 mm, SD = 0.0000), although it was not statistically distinct from the 75% extract (9.00 mm, SD = 1.732). In plain language, these data demonstrate that increasing the concentration of *Capsicum frutescens* leaf extract does improve its antibacterial activity, but the highest observed mean (13.33 mm) remains well below the 25 mm zone of inhibition produced by Imipenem, highlighting the leaf extract's inferior potency relative to a standard antibiotic. From a research perspective, this finding addresses the earlier question of whether varying concentrations generate distinct inhibitory effects and whether any could rival a conventional treatment.

While there is a clear dose-dependent trend—100% being distinctly more effective than the lower concentrations—none approached Imipenem's benchmark, suggesting that, in a clinical context, *Capsicum frutescens* leaf extract lacks the capacity to serve as a standalone therapy.

This outcome resonates with previous studies indicating that leaf phytochemical concentrations are often lower than those in fruits (Moghadam et al., 2023), and that *Acinetobacter baumannii* employs robust outer membranes, efflux pumps, and biofilm formation to ward off natural antimicrobials (Shahin et al., 2025; Shahryari et al., 2021). Furthermore, the use of a single-solvent maceration method (95% ethanol at 60°C) may compromise key bioactive compounds, aligning with research advocating more advanced extraction techniques (Ganić et al., 2022; Guo et al., 2021). Notably, the 75% concentration displayed a numerically higher mean inhibition (9.00 mm) than 50% and 25% (6.00 mm) but did not differ statistically, possibly due to variability in semi-solid extract distribution or partial loss of thermolabile compounds. Consequently, even though higher concentration fosters better inhibition, it still falls short of replicating the potent effect of a well-established antibiotic.

As proposed in Chapter 1, *Capsicum frutescens* leaf extracts might eventually serve as a supportive agent if combined with antibiotics or enhanced by nanoemulsion or encapsulation, potentially mitigating dosage requirements or slowing resistance (Jeong et al., 2023); however, the present findings confirm a substantial performance gap relative to Imipenem's 25 mm zone of inhibition. Overall, these results underscore a statistically significant but practically limited inhibitory capacity for the leaf extracts, illustrating that while concentration influences efficacy, current formulations cannot replace conventional antibiotic therapy against *Acinetobacter baumannii*. The results underscore the necessity for further research to explore potential modifications or synergistic combinations that could enhance the efficacy of the leaf extract.

The results displayed in Tables 1, 2, and 3 highlight a clear dose-dependent trend among the four concentrations of *Capsicum frutescens* leaf extract: 25%, 50%, 75%, and 100%. Although the 100% extract consistently demonstrated the highest inhibition zones (14 mm, 13 mm, 13 mm), it still fell noticeably short of the 25 mm zone produced by the Imipenem disc (positive control). This discrepancy suggests that while *Capsicum frutescens* leaf extracts exert some antibacterial effect, they do not yet approximate the potency of a standardized antibiotic regimen.

In conclusion, while the 100% leaf extract stands out as the most effective among the plant-based treatments, but none of the extracts can match the 25 mm inhibition zone exhibited by Imipenem. These findings align with the concerns raised in Chapter 1 regarding the urgent need for novel antimicrobials to combat *Acinetobacter baumannii*. The present study reaffirms that although *Capsicum frutescens* leaf extracts show promise, additional research on extraction optimization, formulation refinement, and synergy testing will be essential before they can be considered a viable, front-line alternative to established antibiotics.

IV. CONCLUSION

Based on the salient findings of the study, the following conclusions were drawn:

1. The findings demonstrate that higher concentrations (notably 100%) of *Capsicum frutescens* leaf extract generally produce greater zones of inhibition, supporting a dose-dependent effect. Nevertheless, none of these extracts rivaled the potency of the Imipenem disc, indicating that, under current conditions, *Capsicum frutescens* leaf extracts do not match the efficacy of a proven antibiotic.
2. The result suggests that while increasing concentration improves antibacterial activity, none of the extracts approach the effectiveness of the positive control (Imipenem), which indicates a higher performance gap between the natural extracts and established antibiotics.
3. Although the maximum observed antibacterial effect is at 100% *C. frutescens* leaf extract it remains inadequate compared to the 25 mm zone of inhibition achieved by Imipenem. Even though there is a clear dose-dependent trend, the *Capsicum frutescens* leaf extracts, in their current form, cannot serve as a standalone treatment alternative to conventional antibiotics against *Acinetobacter baumannii*.

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