BACOLOD TAY TUNG HIGH SCHOOL

BTTHS JOURNAL OF STUDENTS' PROJECTS
IN ADVANCING RESEARCH KNOWLEDGE

s.p.a.r.k

Volume 1
April 2025

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

Volume 1 *April 2025*

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

Volume 1 *April 2025*

ABOUT THE JOURNAL

The BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K), published annually, symbolizes the bright beginning of student research at Bacolod Tay Tung High School, Inc. This academic, multidisciplinary journal is reviewed by faculty and experts and serves as a platform for the timely publication of outstanding student research. BTTHS Journal of S.P.A.R.K not only documents but also celebrates the research contributions of students presented during the Annual Research Colloquium.

Published by: Bacolod Tay Tung High School, Inc. (描戈律大同中学)
Address: P. Hernaez Street, Bacolod City Negros Occidental, Philippines 6100
Tel No.: (034) 433 2702
Email: research@btths.edu.ph
Website: www.btths.edu.ph

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

Volume 1 *April 2025*

TABLE OF CONTENTS

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

Volume 1 *April 2025*

EDITORIAL

NURTURING TAY TUNGIAN RESEARCH CULTURE

Kristian Brain B. Lecaniel, LPT, RPm
Editor-in-Chief

Bacolod Tay Tung High School, Inc. stands at a pivotal moment in its academic journey, poised to transform from a nurturing ground of intellectual curiosity into a beacon of student-led discovery. The launch of *BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)* marks more than just the inauguration of a school journal—it signals the birth of a vibrant research culture rooted in inquisitiveness, collaboration, and scholarly rigor. By giving students a dedicated platform to publish their inquiries, Tay Tung asserts its commitment to empowering young minds to ask bold questions and pursue evidence-based answers, fostering an environment where research is not an occasional project but a sustained communal pursuit.

At the heart of this emerging culture lies the principle that every student, regardless of background or prior exposure, possesses the potential to contribute meaningfully to knowledge creation. *SPARK* will serve as both catalyst and conduit: catalyst by inspiring learners to transform classroom concepts into real-world investigations, and conduit by channeling their findings into a published format that invites feedback from peers, mentors, and the wider academic community. Workshops on research methodology, mentorship pairings with faculty advisors, and peer review circles will underpin the journal's activities, ensuring that each submission not only reflects individual effort but also benefits from collaborative refinement. In nurturing these practices, Tay Tung cultivates critical thinking, resilience in the face of complex problems, and a lifelong respect for the scientific process.

As *SPARK* lights the way forward, Bacolod Tay Tung High School, Inc. embarks on a collective journey toward excellence in research. The journal's first issue will be more than a compilation of student papers; it will be a testament to the school's evolving identity as a community where inquiry thrives, and young scholars are both architects and beneficiaries of knowledge. By embedding research into the fabric of school life, Tay Tung ensures that each generation of Tay Tungian not only learns about discoveries but becomes an integral part of making them. In doing so, *SPARK* heralds a new era; one in which the flame of inquiry, once kindled, illuminates paths yet untraveled.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

EFFECTIVENESS OF HOMEMADE VACUUM AUTOCLAVE SYSTEM IN STERILIZING GLASS LABORATORY APPARATUSES WITH GRAM-POSITIVE BACTERIA

Mike Eric M. Balontong[1], Jacob D. Flores[2], Samantha Andrea A. Lamata[3], Tristan O. Seruelo[4], Marc Angelo D. Ty[5]

[1 2 3 4 5]Bacolod Tay Tung High School, Inc., Bacolod City, Philippines

mike.balontong@btths.edu.ph , jacob.flores@btths.edu.ph , samantha.lamata@btths.edu.ph , tristan.seruelo@btths.edu.ph , marc.ty@btths.edu.ph

Abstract

Vacuum autoclaves are steam sterilizers that guarantee the absence of microbes to ensure safety and prevent contamination. However, most laboratories have not adopted them due to their high costs and niche purpose. This paper aimed to determine the effectiveness of a homemade vacuum autoclave system in sterilization. Moreover, this study utilized quantitative research as its overall research design with an experimental research approach. The researchers sterilized Petri dishes stained with *Staphylococcus aureus* using a homemade autoclave with and without a vacuum and a commercial autoclave, each with three (3) trials. Samples from each Petri dish were then taken to undergo a Colony Forming Unit (CFU) count to determine the sterilization effectiveness. The data were analyzed through descriptive statistics to determine the mean and standard deviation of the CFU/mL. Inferential statistics evaluated the significant difference between the three aforementioned groups. The homemade autoclave with the vacuum system showed results with highly effective levels of sterilization, with a mean and standard deviation of 0.000 CFU/mL. Similarly, the one without vacuum produced results with highly effective levels, with a mean of 1.210 CFU/mL and a 1.984 standard deviation. Furthermore, the commercial autoclave trials produced a mean of 0.031 CFU/mL and a standard deviation of 0.027. A significant difference was found between the trials done with a homemade autoclave and those without a vacuum. In contrast, no significant difference was found between the trials of all three groups. The results indicated that a homemade autoclave with a vacuum was more effective at sterilization than one without a vacuum. Generally, the homemade vacuum autoclave and commercial autoclave performed similarly, indicating that a homemade vacuum autoclave was just as effective as a commercial autoclave.

Keywords: Homemade autoclave, commercial autoclave, sterilization, *Staphylococcus aureus*, and Colony-Forming Unit (CFU)

1. Background of the Study

Sterilization eliminates all microorganisms; this actively prevents contamination and is essential in surgical operations, pathogen handling, and laboratory experiments (Moran, 2018). There are many methods of sterilization, including using heat through an autoclave (Westlab, 2023). An autoclave is a machine that sterilizes equipment through heat and pressure; a vacuum autoclave uses a vacuum chamber (Tuttnauer, 2024). An autoclave device sterilizes with steam, destroying microorganisms with moist heat through steam (Sastri, 2022). Vacuum autoclaves tend to have low initial purchase prices; however, steep maintenance and operating costs and a niche purpose prevent most laboratories—especially those found in educational institutions—from adopting them (TOMY, 2023). Internationally, steam sterilization is widespread in medical settings due to its safety, ease of use, and effectiveness (Compliance Quest, 2023). However, due to its use of moist heat, it can only be used for non-heat-sensitive materials. Temperature and pressure conditions affect the sterilization process and vary according to the intended results; however, the temperature ranges typically from 121°C to 135°C to be hot enough for sterilization (Gupta, 2024). Autoclaves are also used in other fields; a design by Garf (2021) uses an autoclave made from a repurposed drum as a sterilizer for mushroom growing. A study conducted in the Philippines by Pamplona et al. (2023) concluded that steaming best sterilizes PTC media in remote places, particularly at home (Pamplona et al., 2023). More local research is needed on the effectiveness of a homemade autoclave system, as more research is needed on homemade autoclaves in particular. However, oil-

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

based thermal disinfection technology is being developed as a lower-cost alternative to autoclaves; it operates under principles similar to those of a traditional autoclave: using heat from a liquid to eliminate microorganisms (DailyGuardian, 2023). Few studies on vacuum autoclaves made from recycled materials are available. Even fewer studies assess the effectiveness of homemade vacuum autoclaves in sterilizing laboratory apparatuses with gram-positive bacteria. Furthermore, more local research is needed to evaluate the effectiveness of a homemade vacuum autoclave. This study assessed the effectiveness of homemade vacuum autoclaves in sterilizing gram-positive bacteria, specifically *Staphylococcus aureus*. It intended to provide data on homemade vacuum autoclaves and develop a design that could be compared with commercial autoclaves.

2. Statement of the Problem

Generally, this study aimed to determine the effectiveness of a homemade vacuum autoclave system in sterilization.

Specifically, the researchers sought to answer the following research questions:

1. What is the level of sterilization effectiveness of a homemade vacuum autoclave system in terms of average colony-forming units per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria in the following conditions: a. with the use of a vacuum chamber, and b. without the use of a vacuum chamber?
2. What is the level of sterilization effectiveness of a commercial autoclave system in terms of average colony-forming units per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria?
3. Is there a significant difference between the level of sterilization effectiveness of a homemade vacuum autoclave system in terms of average colony-forming unit per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria with and without a vacuum chamber?
4. Is there a significant difference between the level of sterilization effectiveness of a homemade and commercial vacuum autoclave system in terms of average colony-forming unit per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria?

3. Framework of the Study

Overall, three theories—the Vacuum Theory, the Germ Theory of Disease, and the Sterilization theory—supported the effectiveness of a homemade vacuum autoclave system in sterilizing glass laboratory instruments. These theories served as the foundation for this research.

To create a vacuum, one must first understand the underlying principles of what produces a vacuum. The Vacuum Theory outlines the fundamentals of vacuums and how these concepts are applied to the design and construction of vacuum chambers. Boyle's Law states that in an environment where the temperature remains constant, the relationship between a gas's pressure and volume is inversely proportional (Benson, 2021). Per the law, if a sealed container were to expand and increase its volume, the pressure inside would decrease. This produces a low-pressure area, creating a vacuum (Vac Aero, 2018). Items and substances placed inside vacuum chambers behave differently due to environmental change. Liquids vaporize into gases due to the lowered pressure, which affects the liquid's vapor pressure. As for solids, items such as a deflated balloon will expand due to the relationship between pressure and volume; as the vacuum starts, the object's volume increases (Marzouk et al., 2018). The vacuum theory supports the study by providing knowledge of the possible effects, occurrences, and explanations when working with vacuums. This information was used to properly construct the vacuum chamber by applying the laws of pressure and vacuum to assemble a successful and efficient vacuum autoclave.

The Germ Theory of Disease states that diseases are caused by germs and organisms too small to be seen by the naked eye (Britannica, 2024). This theory was initially developed in the mid-19th century by French chemist Louis Pasteur, who challenged Aristotle's archaic Theory of Spontaneous Generation, which states that life can emerge from non-living matter, providing reasons as to why decomposers such as grubs and worms may seem to appear on spoiled food (Comunale, 2023) suddenly. However, Pasteur disproved this theory through multiple successful experiments that proved spoilage and the emergence of microorganisms through extreme exposure to outer factors, with his most famous being the Swan-neck flask experiment in 1861 (Carr, 2020). The Germ Theory of Disease was further expanded on by German physician Robert Koch in the late 19th century, who established that germs and other microorganisms could enter hosts and cause certain diseases, forming his four postulates that are used to determine the exact type of pathogen causing a particular illness (Moriarty et al., 2020). This theory supported the researchers by providing a foundation for microbiology, insights into germs and how they infect through contact with a host, and how to eliminate them.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Sterilization is any process that involves the elimination of all microorganisms on a surface or solution (Cherney, 2021). There are many types of sterilization, including moist heat sterilization, dry heat sterilization, gas sterilization, sterilization by radiation, and sterilization by filtration (Kerone, 2021). Heat destroys microbial life through denaturation. Denaturation is a process in which the structures of proteins are broken and disrupted, rendering bacteria and other microorganisms unable to cause infection (Butreddy et al., 2021). Moist heat sterilization, also known as autoclaving, hastens the process by adding moisture and utilizing steam, making it one of the most effective sterilization methods. However, steam at atmospheric air pressure would not be enough for sterilization. Gay-Lussac's law states that if a gas's volume and amount are held constant, its pressure will become directly proportional to its temperature and vice versa (Let's Talk Science, 2020). This means that increasing the pressure in a vacuum autoclave would help to improve the temperature to a sufficient level and increase the boiling point to a heat where sterilization is effective (Sapkota, 2021). This theory guided the researchers in building an autoclave that can effectively sterilize by applying the rules that make sterilization possible.

4. Significance of the Study

This study's results provided information and knowledge regarding the effectiveness of a homemade vacuum autoclave in sterilizing glass laboratory instruments with gram-positive bacteria. The following may benefit from the valuable information gained from the results and findings of this study:

Students. This study can help students by giving them knowledge and the ability to sterilize glass laboratory equipment with a homemade vacuum autoclave. These experiences encourage scientific curiosity and creativity, enabling students to test other materials and improve their ideas. Additionally, the availability of laboratory sterilization equipment better protects the students' hygiene.

Teachers. This study will help teachers with an inexpensive homemade vacuum autoclave design to be built for laboratory use. Conducted experiments, especially the ones biological in nature, will be far more hygienic and sterile.

School Administrators. The results of this study allow School Administrators to supply and use a cheap and effective alternative vacuum autoclave system for the institution. This maintains high safety standards at a reasonable cost by ensuring all tools are properly sterilized.

Academic Institutions. Financial limitations are common for academic institutions, mainly when investing in modern lab equipment. The project allows school systems to develop less expensive alternatives to pricey vacuum autoclaves, enabling them to adhere to budgetary constraints while upholding high education standards.

Laboratory Personnel. This study will further develop laboratory sterilization methods and provide an inexpensive alternative design for a vacuum autoclave made from recycled materials.

Healthcare Professionals. This study can benefit healthcare professionals, such as doctors and medical technologists, particularly in resource-limited environments, by providing a reliable, accessible method for sterilizing instruments.

Future Researchers. This study can benefit those who are interested in conducting similar studies. The results and findings can be used in their papers.

5. Scope and Limitations

This study described and compared the effectiveness of a homemade vacuum autoclave system in sterilizing glass laboratory apparatuses with gram-positive bacteria when autoclaving with and without a vacuum. Moreover, the researchers only used the colony forming unit per mL (CFU/mL) to determine the level of sterilization, as the study only aimed to determine whether or not the autoclave functions effectively and only tested one (1) type of gram-positive bacteria, namely *S. aureus*, due to financial and time constraints. Furthermore, only three (3) trials were done per test: three (3) trials for the autoclave system with the vacuum chamber, three (3) trials without the vacuum chamber, and three (3) trials for the control group—the commercially available autoclave. Lastly, due to the researchers' lack of experience, the experiment was tested and run at a Medical Technology Laboratory in an Autonomous University in Bacolod City with the assistance and supervision of Medical Technologists. Lastly, the data was then studied and analyzed at Bacolod Tay Tung High School, Inc., during the School Year 2024-2025.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

6. Literature Review

Vacuum Autoclave Sterilization. Autoclaves and autoclaving are sterilization processes; as such, they are used in settings where sterility is paramount (Toi, 2020). As autoclaves are typically used in environments requiring sterility, their effectiveness often comes into question. However, a few studies implied that the effectiveness of autoclaves had less to do with the process and more to do with the procedures and the operator's education (Panta et al., 2019; Strange, 2024; Mechler, 2024). That being said, the type of autoclave should also be questioned. Vacuum autoclaves have higher steam penetration, killing most pathogens for a more sterile environment (Tuttnauer, 2024). Typically, these autoclaves are set to run at 121°C with 15 psi for 20 mins; these conditions dehydrate microbe cells (Elkhatat, 2022).

Sterilizing Gram-Positive Bacteria. Gram-positive bacteria are part of the genus family and are members of the Phylum Firmicutes. They started the color of crystal violet when stained in gram stain testing, which resulted in a gram-positive outcome (Sizar et al., 2023). After multiple studies, it was concluded that gram-positive bacteria have a thick peptidoglycan layer (Rowden, 2022). Bacteria can be killed or deactivated through hot steam, such as autoclaves, UV rays, and chlorine exposure, but some are resistant to specific methods (Chen et al., 2023). Scorching temperatures can sterilize areas, and autoclaves can perform those conditions under time and pressure. Alcohol is another way of disinfecting bacteria; it is a soluble chemical compound consisting of ethyl or isopropyl. However, the alcohol is only bactericidal and can only kill bacteria (CDC, 2024). Autoclaving, or steam sterilization, also eliminates infectious bacteria and denatured proteins. "Wet heat" sterilizes thoroughly, making it the most dependable method for decontaminating biohazardous waste (US San Diego, 2024).

Colony Forming Unit. A Colony Formation Unit (CFU) is used in the laboratory to count bacteria or fungus samples, specifically in microbiology (News Medical, 2024). A CFU refers to individual microorganism groups. CFU counts are measured as CFU per gram or milliliter (CFU/g or CFU/ml) (Kleinfeld, 2024). CFU is beneficial throughout microbiology for quantifying bacteria, though it has a variety of procedures depending on the specific area of application (Nai et al., 2021). Biologist: developed the first in vivo clonal strategy, later termed the spleen colony-forming unit (CFU-S). This method assessed the functional capacity of immature blood cells, or hematopoietic progenitors, from bone marrow at the single-cell level (Baena et al., 2021). A population of bacteria that developed and matured in a laboratory setting was called a culture, which varied in the number of microorganisms present in the dish. Aseptic workspaces and apparatuses were required to maintain accurate results, with sterilization needed to achieve sterile conditions (Ahern, 2019).

7. Methods

Study Design. This study utilized quantitative research as its overall research design. An experimental research approach was chosen using specific quantitative research approaches, as this study primarily focuses on complex sciences (Bhandari, 2022). According to Saha (2024), experimental research is a systematic and scientific approach in which the researchers manipulate independent variables and observe and record their effects on dependent variables. Furthermore, this study enables the systematic manipulation of the independent variable, sterilization using a homemade vacuum autoclave, and a comparison of its effects on the dependent variable, the sterilization effectiveness in terms of colony forming unit (CFU).

Materials. To develop a homemade vacuum autoclave, the following materials were used pressure cooker, distilled water, ¼ diameter by 3-inch pipe, a brass T-connector, a positive pressure gauge, a negative pressure gauge, a brass nipple, a brass adapter, a brass ball valve, soft PVC tube, AC condenser, hot plate, metal epoxy, Teflon tape. Furthermore, the total cost of the homemade vacuum autoclave was only ₱ 1,487.00.

Equipment. To conduct the experiment, the following pieces of equipment were used: a 500 mL beaker, agar plate, conical flask, foil, hot plate with a magnetic stirrer, incubator, isopropyl alcohol, measuring scale, nutrient agar, parafilm, Petri dishes, personal protective equipment, *Staphylococcus aureus* samples in broth culture, sterile swabs, test tube rack, and timer.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Figure 1
Homemade Vacuum Autoclave

Procedures. The study was conducted in four main stages. During the pre-development stage, the researchers prepared the necessary forms and plans. The development stage saw the creation and initial testing of the homemade vacuum autoclave. In the experimental stage, the researchers stained glass Petri dishes with *S. aureus*, then treated by a homemade autoclave with and without a vacuum and a commercial autoclave. Swab samples were taken from the Petri dishes and were allowed to incubate on nutrient agar. Colony-forming unit (CFU) counting was then performed. The data was collected, arranged, statistically analyzed, and interpreted. Descriptive and inferential statistics were used. Furthermore, all tests for significance were set at a 95% confidence level. Moreover, Table 1 outlined the basis for the level of sterilization effectiveness. The following bacterial growth parameters proposed by Alfy (2022) were used.

Table 1
Interpretation of CFU/mL Results in Terms of Bacterial Growth and Level of Sterilization Effectiveness

CFU/mL	Bacterial Growth Interpretation	Level of Sterilization Effectiveness
0 CFU/mL	No Growth (NG)	Very Effective
< 2 CFU/mL	Scanty Growth (ScG)	Very Effective
> 2-12 CFU/mL	Very Slight Scanty Growth (VSG)	Slightly Effective
> 12-40 CFU/mL	Slight Growth (SG)	Slightly Effective
> 140-100 CFU/mL	Moderate Growth (MG)	Not Effective
> 100-250 CFU/mL	Heavy Growth (HG)	Not Effective
≥ 250 CFU/mL	Very Heavy Growth (VHG)	Not Effective

8. Results and Discussion

Table 2
Average Colony-Forming Units per milliliter (CFU/mL) of Staphylococcus aureus in Homemade Autoclave System With and Without a Vacuum

	Trial	Mean	SD	Interpretation
With Vacuum	0.000 CFU/mL$_1$ 0.000 CFU/mL$_2$ 0.000 CFU/mL$_3$	0.000 CFU/mL	0.000	Highly Effective
Without Vacuum	0.000 CFU/mL$_1$ 3.500 CFU/mL$_2$ 0.130 CFU/mL$_3$	1.210 CFU/mL	1.984	Highly Effective

Note: 1,2,3 indicates the order of trial

Table 2 depicts the results of the trials done with a homemade autoclave with and without a vacuum regarding the bacterial count (*Staphylococcus aureus*) remaining after sterilization. All three trials done with a vacuum yielded 0.000

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

CFU/mL; the mean and standard deviation were also 0.000 CFU/mL and 0.000, respectively. The results indicated that no bacteria remained after the homemade autoclave process with a vacuum. Meanwhile, in the trials done with a vacuum, only the first yielded 0.000 CFU/mL; trials 2 and 3 resulted in 3.500 CFU/mL and 0.130 CFU/mL, respectively. This results in a mean of 1.210 CFU/mL with a standard deviation of 1.984 for the trials without a vacuum; the high standard deviation indicates that values were spread out over a broader range and farther from the mean, while the mean suggested that some bacteria remained after the homemade autoclave process without a vacuum. A homemade autoclave process with a vacuum produced results with no bacterial growth, while the process without a vacuum only produced results with scanty bacterial growth. This implied that both methods resulted in high levels of effectiveness or are highly effective. The difference in their means indicated that a vacuum chamber enhances sterilization better than without a vacuum.

A study by Tuttnauer (2020) supports the data, it states that air acts as a barrier that prevents hot steam from having contact; thus, removing air can be beneficial; it leaves the chamber with all the free space for the hot steam and can sterilize effectively, especially apparatuses with hollow or porous designs. This suggests that vacuum autoclaves have higher steam penetration, killing most pathogens for a more sterile environment. Thus, the presence of a vacuum results in a sterilization level better than that of a non-vacuum autoclave.

Table 3

Average Colony-Forming Units per milliliter (CFU/mL) of Staphylococcus aureus in Commercial Autoclave System

	Trial	Mean	SD	Interpretation
Commercial	0.050 CFU/mL$_1$ 0.043 CFU/mL$_2$ 0.000 CFU/mL$_3$	0.031 CFU/mL	0.027	Highly Effective

Note: 1,2,3 indicates the order of trial

Table 3 depicted the results of the trials done with a commercial autoclave regarding the bacterial count (*Staphylococcus aureus*) remaining after sterilization. Trials 1 and 2 yielded 0.050 CFU/ml and 0.043 CFU/mL, respectively, while trial 3 resulted in 0.000 CFU/mL. The average bacterial count (*S. aureus*) remaining after sterilization with the commercial autoclave system is 0.031 CFU/mL, with a standard deviation of 0.027. The results indicated that few bacteria remained after the commercial autoclave process. It can be interpreted that a commercial autoclave process with a vacuum produced results with scanty bacterial growth, thereby indicating a highly effective level of sterilization.

A study by Iqbal (2023) found that commercial autoclaves yield quality control of sterilization by creating a high-temperature, high-pressure environment, a lethal setting for most forms of bacterial life. The study supports the data that the commercial autoclave ensured proper sterilization using the standard parameters of 15 PSI, at least 121°C, and 20 mins. Thus, a commercial autoclave is efficient in maintaining sterile results.

Table 4

Difference Between the Average Colony-Forming Units per milliliter (CFU/mL) of Staphylococcus aureus in Homemade Autoclave System With and Without a Vacuum

	Test	Statistic	df	p
CFU/mL	Mann-Whitney	NaN		

Note: The variance in CFU/mL is equal to 0 after grouping

The data in Table 4 showed that the group with vacuum resulted from NaN (Not a Number) due to the values equal to zero. Due to the lack of variability in the With Vacuum group (0 SD), a statistical test could not be performed. As such, descriptive statistics were used to compare the two groups further.

Table 5

Descriptive Results of Trials with A Homemade Autoclave with and without Vacuum

	Group	N	Mean	SD	Coefficient of Variation
CFU/mL	W/ Vacuum	3	0.000	0.000	NaN
	W/o Vacuum	3	1.210	1.984	1.6403

Note: The variance in CFU/mL is equal to 0 after grouping

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Table 5 depicts the descriptive results of the trials with a homemade autoclave with and without a vacuum. The mean of the trial done with vacuum resulted in 0.000 CFU/mL, meaning all three trials resulted in complete sterilization with zero bacterial growth. Meanwhile, the results of the trials without vacuum resulted in 1.210 CFU/mL, indicating the presence of scanty bacterial growth. The standard deviation and coefficient of variation for the trials with vacuum were 0.000 and NaN, respectively; this suggested no variation at all, meaning all three trials had the same results. On the other hand, the standard deviation and coefficient of variation for the trials without vacuum were 1.984 and 1.640, respectively. The standard deviation was relatively high compared to the mean, and the high coefficient of variation indicates a high variability in the results of the trials. This suggested that homemade autoclave processes without a vacuum could be unreliable or inconsistent. Overall, the descriptive statistics indicated a significant difference between the level of sterilization effectiveness of a homemade vacuum autoclave system in terms of CFU/mL in autoclaving *S. aureus* bacteria with and without a vacuum chamber. The trials done with the vacuum showed more significant levels of sterilization effectiveness.

A study from RAYPA (2022) supports the data that vacuum autoclaves are more favorable in achieving an effective decontamination procedure. The vacuum autoclave system eliminates air barriers around the apparatuses, allowing guaranteed steam penetration across all surfaces. A non-vacuum autoclave system only requires high hot steam moisture to operate with standard parameters. Without air removal, the efficiency of a non-vacuum autoclave is significantly different from that of a vacuum autoclave system. Thus, vacuum autoclaves are far more efficient than non-vacuum autoclaves in sterilizing laboratory apparatuses.

Table 6

Difference Between the Average Colony-Forming Units per milliliter (CFU/mL) of Staphylococcus aureus in Homemade and Commercial Autoclave Systems

	Statistic	df	p	Interpretation
Kruskal-Wallis Test	3.307	2	0.191	Not Significant

Note: The variance in CFU/mL is equal to 0 after grouping

Table 6 depicted the results of a non-parametric Kruskal-Wallis test between trials with a homemade autoclave with a vacuum, without a vacuum, and a commercial autoclave. The p-value of 0.191 was more significant than the $\alpha = 0.05$ (since the confidence level is 95%). Therefore, there was no significant difference between the level of sterilization effectiveness of a homemade autoclave system in terms of CFU/mL in *S. aureus* bacteria with a homemade autoclave with a vacuum, without a vacuum, and a commercial autoclave. Furthermore, the With Vacuum setup performed similarly to the Commercial autoclave, suggesting it is just as effective.

The data was supported by the study of Elkhatat (2022) in terms of decontamination processes in several applications. Moist heat sterilization kill pathogens due to high energy. These extreme conditions kill the microbes by dehydrating their cells. Despite the different types of autoclaves compared in the research study, the setups can still sterilize apparatuses but only differ in their effectiveness. All the autoclaves mentioned in the data followed all the standard parameters in sterilizing apparatuses.

9. Conclusions and Recommendations

Conclusions

The findings of this study confirmed the claims of various research papers regarding the qualities of homemade and commercial autoclaves and standard parameters for sterilizing laboratory apparatuses with gram-positive bacteria.

First, the results indicated that the level of sterilization effectiveness of a homemade vacuum autoclave system with a vacuum in terms of average colony-forming units per milliliter (CFU/mL) in autoclaving *Staphylococcus aureus* bacteria was high due to the absence of bacterial growth; it reached 100% sterilization with all trials resulting in 0.000 CFU/mL. This revealed that the correct standard perimeter for autoclaving gram-positive bacteria was present in the homemade vacuum autoclave system with a vacuum, thus indicating that the homemade vacuum autoclave ran with a vacuum was capable and highly effective in sterilizing gram-positive bacteria. Meanwhile, measuring the sterilization effectiveness of a homemade vacuum autoclave system without a vacuum in terms of average colony-forming units per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria resulted in scanty bacterial growth, thus indicating a highly effective level of sterilization. Its trials' mean CFU/mL resulted in 1.210 CFU/mL, a small amount commonly considered negligible. This indicated that the conditions were met for sterilization; however, the absence of a vacuum

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

prevented complete sterilization. Still, the homemade vacuum autoclaves that ran with and without a vacuum were capable and highly effective in sterilizing gram-positive bacteria.

Secondly, the results indicated that the level of sterilization effectiveness of a commercial autoclave system in terms of average colony-forming units per milliliter (CFU/mL) in autoclaving *S. aureus* bacteria was high due to producing results with scanty bacterial growth; its trials garnered a mean of 0.031 CFU/mL. It should be noted that such a small amount is considered negligible. Furthermore, a standard deviation of 0.027 indicated precise results. Therefore, the commercial autoclave was highly effective in sterilizing gram-positive bacteria.

Thirdly, the difference between the average colony-forming units per milliliter (CFU/mL) of *S. aureus* in the homemade autoclave system with and without a vacuum resulted in NaN (Not a Number) when undergoing statistical analysis; this was because the mean value of the trials done with vacuum resulted in 0.000 CFU/mL. Descriptive statistics were utilized instead to counteract this. It was found that the trials done with a vacuum resulted in a mean of 0.000 CFU/mL and a standard deviation of 0. On the other hand, the trials done without a vacuum resulted in a mean of 1.210 CFU/mL and a standard deviation of 1.984. While both processes can sterilize, the results concluded a significant difference between the average colony-forming units per milliliter (CFU/mL) of *S. aureus* in the homemade autoclave system with and without a vacuum. Therefore, the first hypothesis was rejected, as a significant difference existed between the trials with and without a vacuum. Furthermore, the data provided implies that a vacuum chamber enhances sterilization better than without a vacuum.

Finally, after a non-parametric Kruskal-Wallis test, the difference between the average colony-forming units per milliliter (CFU/mL) of *S. aureus* in homemade and commercial autoclave systems resulted in a p-value of 0.191. As tests were set at a 95% confidence level, the p-value indicated no significant difference between the average colony-forming units per milliliter (CFU/mL) of *S. aureus* in homemade and commercial autoclave systems. Thus, the second null hypothesis was accepted. This indicated that all autoclave systems sterilized their prospective apparatuses well enough to receive a low enough mean CFU/mL to be considered negligible. Although a difference in sterilization effectiveness was present between the three systems, it was deemed non-significant. Therefore, the homemade vacuum performed just as well as the commercial one.

Recommendations

Future researchers can further investigate the effectiveness of homemade vacuum autoclaves against other gram-positive bacteria or their effectiveness against gram-negative bacteria. To continue fueling the spirit of innovation, they may also create their design of a homemade autoclave using other recycled parts and compare different parameters. They may also opt to conduct more sterilization trials for a more accurate result or conduct CFU counting before and not just after the trials to measure the percent reduction.

10. Declaration of Conflict of Interest

Authors have declared that no competing interests exist.

11. Literatures Cited

Ahern, H. (2019). Bacteriological culture methods. Milne Library Publishing. https://milnepublishing.geneseo.edu/suny-microbiology-lab/chapter/bac teriological-culture-methods/

Alfy, M. (2022). Assessing decontamination practices at a medical microbiology research laboratory. Journal of Biosafety and Biosecurity. https://www.sciencedirect.com/science/article/pii/S2588933822000218

Baena, A. R. y, Manso, B. A., & Forsberg, E. C. (2021). CFU-S assay: a historical single-cell assay that offers modern insight into clonal hematopoiesis. Experimental Hematology, https://doi.org/10.1016/j.exphem.2021.10.003

Benson, T. (2021). Animated Boyle's Law. NASA. https://www.grc.nasa.gov/www/k-12/airplane/aboyle.html

Bhandari, P. (2022, April 4). What is quantitative research? | Definition & methods. Scribbr. https://www.scribbr.co.uk/research-methods/introduction-to-quantitative-research/

Britannica. (2024, November 5). Germ https://www.britannica.com/science/germ-theory

Butreddy, A., Janga, K. Y., Ajjarapu, S., Sarabu, S., & Dudhipala, N. (2021). Instability of therapeutic proteins — An overview of stresses, stabilization mechanisms and analytical techniques involved in lyophilized proteins. International Journal of Biological Macromolecules, https://doi.org/10.1016/j.ijbiomac.2020.11.188

Carr, S. M. (2020). Pasteur Swan-Neck flask experiment (1861): Experimental disproof of Spontaneous https://www.mun.ca/biology/scarr/4270_Pasteur_experiment.html

CDC. (2024, March 11). Sterilization. Infection Control. https://www.cdc.gov/infection-control/hcp/disinfection-

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

sterilization/sterilization.html
Chen, X., Chen, Z., Ngo, H. H., Mao, Y., Cao, K., Shi, Q., Lu, Y., & Hu, H.-Y. (2023). Comparison of inactivation characteristics between Gram-positive and Gram-negative bacteria in water by synergistic UV and chlorine disinfection. Environmental Pollution, 333, 122007. https://doi.org/10.1016/j.envpol.2023.122007
Cherney, K. (2021, February 26). The difference between disinfecting and sterilizing. Healthline. https://www.healthline.com/health/disinfect-vs-sterilize
Compliance Quest. (2023). Sterilization in healthcare | Best practices & standards. ComplianceQuest. https://www.compliancequest.com/a-z-glossary/sterilization/
Comunale, J. (2023, November 23). Spontaneous generation theory | Definition & examples. Study.com. https://study.com/learn/lesson/spontaneous-generation-theory-examples.html
DailyGuardian. (2023, October 20). TMC Iloilo, UPV ink partnership for more efficient hospital waste management. Daily Guardian. https://dailyguardian.com.ph/tmc-iloilo-upv-ink-partnership-for-more-efficient-hospital-waste-management/
Elkhatat, A. M. (2022). Recent trends of microbial decontamination for occupational, industrial and domestic applications. Bulletin of the National Research Centre, 46(1). https://doi.org/10.1186/s42269-022-00761-9
Garf. (2021, May 7). How to build a drum steriliser for mushroom growing. Archer's Mushrooms. https://archersmushrooms.co.uk/how-to-build-a-drum-steriliser-for-mushroom-growing/
Gupta, A. (2024). Sterilization 101: How does a laboratory autoclave work? Consolidated Sterilizer Systems. https://consteril.com/how-does-a-laboratory-autoclave-work/
Iqbal, F. (2023, August 15). LinkedIn. Linkedin.com. https://www.linkedin.com/pulse/ensuring-product-integrity-power-autoclave-qualty-control-iqbal
Kerone. (2021, February 4). Different types of sterilization process. Kerone Engineering Solutions Limited. https://kerone.com/blog/different-types-of-sterilization-process/
Kleinfeld, H. (2024, January 23). Colony forming units — What are they and how many do you need? Omni-Biotic Probiotics. https://omnibioticlife.com/blogs/blog/what-are-colony-forming-units
Let's Talk Science. (2020, January 20). Charles' law and Gay-Lussac's law. https://letstalkscience.ca/educational-resources/backgrounders/charles-law-and-ga y-lussacs-law
Marzouk, O. A., Jul, W. a. M. H. R., Jabri, A. M. K. A., & Al-Ghaithi, H. a. M. A. (2018). Construction of a small-scale vacuum generation system and using it as an educational device to demonstrate features of the vacuum. International Journal of Contemporary Education, 1(2). https://doi.org/10.11114/ijce.v1i2.3554
Mechler, S. (2024, August 19). 7 Common Autoclave Problems You Should be Aware Of. Consolidated Sterilizer https://consteril.com/common-autoclave-problems/
Moran, S. (2018). An applied guide to water and effluent treatment plant design. ScienceDirect. https://www.sciencedirect.com/topics/chemistry/sterilization-and-disinfection
Moriarty, T., Leggat, W., Hugget, M.J., & Ainsworth, T.D. (2020). Coral disease causes, consequences, and risk within coral restoration. Trends In Microbiology. 28(10), 793-807. https://doi.org/10.1016/j.tim.2020.06.002
Nai, G. A., Martelli, C. a. T., Medina, D. a. L., De Oliveira, M. S. C., Caldeira, I. D., Henriques, B. C., Portelinha, M. J. S., Eller, L. K. W., & Marques, M. E. A. (2021). Fractal dimension analysis: A new tool for analyzing colony-forming units. https://doi.org/10.1016/j.mex.2021.101228
News Medical. (2024, June 20). What is a colony forming unit? News Medical Life Sciences. https://www.news-medical.net/whitepaper/20240620/What-is-a-colony-f orming-unit.aspx
Pamplona, R. S., Dela Cruz, C. DV., & Tayangona, M. A. (2023). Selected laboratory equipment and kitchen utensils as an alternative sterilization method for sterilizing plant https://doi.org/10.1101/2023.10.26.564287
Panta, G., Richardson, A. K., & Shaw, I. C. (2019). Effectiveness of autoclaving in sterilizing reusable medical devices in healthcare facilities. The Journal of Infection in Developing Countries, 13(10), 858–864. https://doi.org/10.3855/jidc.11433
RAYPA. (2024, November 21). Pre vacuum autoclaves | RAYPA. RAYPA. https://www.raypa.com/en/types/pre-and-post-vacuum-autoclaves/
Rowden, A. (2022, September 29). What is the difference between gram-positive and gram-negative bacteria? Medical News Today. https://www.medicalnewstoday.com/articles/gram-positive-vs-gram-negative#treatment
Saha, S. (2024, June 16). What is experimental research: Definition, types & examples. https://www.entropik.io/blogs/experimental-research
Sapkota, A. (2020, May 11). Physical methods of sterilization- Heat, filtration, radiation. Microbe

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

https://microbenotes.com/physical-methods-of-sterilization/
Sastri, V. R. (2022). Material requirements for plastics used in medical devices. Plastics in Medical Devices, https://doi.org/10.1016/b978-0-323-85126-8.00008-4
Sizar, O., Unakal, C. G., & Leslie, S. W. (2023, May 30). Gram positive bacteria. StatPearls. StatPearls https://www.ncbi.nlm.nih.gov/books/NBK470553/
Strange, M. (2024, August 23). Proper loading of autoclaves: the dos and don'ts. Tuttnauer. https://tuttnauer.com/blog/proper-loading-autoclaves-dos-and-donts
Toi, T. (2020, September 12). Autoclaves: Principles, Uses, Types, and Procedures. MES. https://www.mesaustralia.com.au/blogs/news/autoclaves-principles-use s-types-procedures
TOMY. (2023, September 27). The complete guide to autoclave prices: unveiling the true cost of https://tomy.amuzainc.com/blog/autoclave-price/
Tuttnauer. (2020, November 4). How Does a Non-Vacuum Process Contribute to Sterilization Failure? Tuttnauer.com; Tuttnauer. https://tuttnauer.com/blog/how-does-non-vacuum-process-contribute-sterilization-failure
Tuttnauer. (2024). Autoclave sterilization process guide. https://tuttnauer.com/blog/autoclave
UC San Diego. (2024, March 2). Autoclave Overview. UC San Diego. https://blink.ucsd.edu/safety/research-lab/biosafety/autoclave/index.ht ml
Vac Aero International. (2018, August 10). The fundamentals of vacuum theory. VAC AERO International Inc. https://vacaero.com/information-resources/vac-aero-training/170466-the-fundame ntals-of-vacuum-theory.html
Westlab. (2023, May 2). Different sterilization methods used in the laboratory. Westlab. https://www.westlab.com/blog/different-sterilization-methods-used-in-th e-laboratory

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

ANTIBACTERIAL EFFICACY AND CYTOTOXICITY OF BLUE TERNATE (*Clitoria ternatea*) FLOWER ETHANOLIC EXTRACT USING MINIMUM INHIBITORY CONCENTRATION (MIC) AND BRINE SHRIMP (*Artemia salina*) LETHALITY ASSAY (BSLA)

Erin Joie G. Agraviador[1], Ella Casandra H. Borromeo[2], Elphie Grace A. Cahilig[3], Richard D. Labuson[4], Charmaine Ann C. Lim[5], Paige G. Paypon[6], Chelsea Anne S. Sia[7]

[1 2 3 4 5 6 7]Bacolod Tay Tung High School, Inc., Bacolod City, Philippines

erin.agraviador@btths.edu.ph , ella.borromeo@btths.edu.ph , elphie.cahilig@btths.edu.ph , richard.labuson@btths.edu.ph , charmaine.lim@btths.edu.ph , paige.paypon@btths.edu.ph , chelsea.sia@btths.edu.ph

Abstract

Bacteria are ancient and diverse microorganisms, with most being harmless or beneficial. However, the rise of antimicrobial resistance has rendered many antibiotics ineffective, necessitating the search for alternative treatments. This study aimed to evaluate the antibacterial efficacy and cytotoxicity of *Clitoria ternatea* flower ethanolic extract. A true-experimental research design was employed, utilizing the Minimum Inhibitory Concentration (MIC) test to determine the lowest extract concentration that inhibited the following gram-positive and gram-negative bacteria, namely *Staphylococcus aureus* (gram-positive) and *Klebsiella pneumoniae* (gram-negative). The Brine Shrimp Lethality Assay (BSLA) assessed its cytotoxicity against Brine Shrimp (*Artemia salina*). The MIC test confirmed the antibacterial activity of the extract in which clear and translucent findings can be observed across various concentrations. The BSLA results showed no significant difference in toxicity across various concentration levels, indicating low toxicity (p value = 0.566). These findings suggested that *C.ternatea* flower ethanolic extract exhibited antibacterial properties while maintaining a favorable safety profile. Thus, this study provided insights into its potential as a natural antibacterial agent and emphasized the need for further investigation into its bioactive compounds.

Keywords: Blue Ternate (*Clitoria ternatea*), antibacterial efficacy, cytotoxicity, Minimum Inhibitory Concentration (MIC), Brine Shrimp (*Artemia salina*) Lethality Assay (BSLA)

1. Background of the Study

Bacteria, the most ancient living species on Earth, exhibit incredible diversity and abundance (Blaser & Philips, 2014). While most bacteria are beneficial to human health, some are harmful, causing mild to severe diseases (Soni & Pandey, 2024). Pathogenic bacteria are those that have the ability to cause disease. A pathogen is an organism that causes illness in a host (Santos-Longhurst, 2019).

Antibiotics, also known as antibacterials, are medications used to combat infections caused by bacteria. Certain strains of bacteria have become resistant to antibiotics, making it difficult to treat infections (Healthline Medical Network, 2023). The growing prevalence of drug-resistant pathogens poses a serious public health issue, necessitating urgent global efforts to develop new treatment strategies and implement effective antimicrobial management (World Health Organization, 2023).

The misuse and overuse of antibiotics in human and animal healthcare, along with poor infection control practices in medical settings, has given rise to a serious worldwide health risk known as antibiotic resistance (Septimus, 2018). Consequently, within the field of biomedical science, there is a strong emphasis on recognizing the value of therapeutic plants (Sibanda & Okoh, 2007). Herbal medicine provides solutions for combating antibiotic resistance (Izah, 2024). Among these, blue ternate (*Clitoria ternatea*) is an adaptable plant used in traditional Ayurvedic treatment. Several beneficial studies on this plant have been conducted throughout the years, revealing it to have a variety of health benefits and so providing a better understanding of its potential use (Shirodkar et.al., 2023). Although several studies

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

have demonstrated its health benefits, research on antibacterial activity and cytotoxicity remains limited. Thus, more research is needed to establish its potential as a natural alternative to conventional antibiotics.

The purpose of this study is intended to investigate the antibacterial efficacy and cytotoxicity concentration of C. ternatea extracts by using the Minimum Inhibitory Concentration test (MIC) and the Brine Shrimp Lethality Assay. Thus, *C. Ternatea* has the potential to be a herbal medication used in the development of novel pharmaceuticals for natural plant-based medication.

2. Statement of the Problem

This study aimed to investigate the antibacterial efficacy and cytotoxicity of the ethanolic extract of blue ternate (*Clitoria ternatea*) flowers through two main assays: the Minimum Inhibitory Concentration (MIC) test and the Brine Shrimp (*Artemia salina*) Lethality Assay (BSLA).

Specifically, the researchers sought to answer the following research questions:

1. What was the Minimum Inhibitory Concentration (MIC) of blue ternate (*C. ternatea*) flower ethanolic extract against the following gram-positive and gram-negative bacteria namely: a. *Staphylococcus aureus* (gram-positive) b. *Klebsiella pneumoniae* (gram-negative) according to the following concentration:
    a.) 0%,
    b.) 25%,
    c.) 50% ,
    d.) 75%, and
    e.) 100%?
2. What was the lethality rate of blue ternate (*C. ternatea*) flower ethanolic extract against brine shrimp (*A. salina*) in the following cytotoxicity concentrations:
    a.) Control Group,
    b.) 0.00106 mg/mL,
    c.) 0.00265 mg/mL,
    d.) 0.00530 mg/mL, and
    e.) 0.01060 mg/mL?
3. Is there a significant difference between the Brine Shrimp (*A. salina*) Lethality Assay (BSLA) of blue ternate (*C. ternatea*) flower ethanolic extract against the following cytotoxicity concentrations:
    a.) Control Group,
    b.) 0.00106 mg/mL,
    c.) 0.00265 mg/mL,
    d.) 0.00530 mg/mL, and
    e.) 0.01060 mg/mL?

3. Framework of the Study

The researchers assessed the antibacterial activity of *C. ternatea* employing the Minimum Inhibitory Concentration test which identified the lowest concentration extract required to inhibit bacterial growth. The primary goal of the BSLA in this study was to determine the cytotoxic effects of the bioactive compounds found in *C. ternatea*. For agricultural uses and other industrial uses, extraction should be done to separate components of butterfly pea. Traditional extraction methods (conventional method) and modern methods (Non-conventional methods) are the types of extractions. (Jeyaraj et al., 2021). The yield of this extraction method depends on various factors like temperature, pH, types of solvent used, & extraction time. Since the 1970s, conventional extraction methods have been maneuvering for blue ternate plants. (Lakshan et al., 2019). There are high chances of various other phytochemicals being present in the extract. Several studies investigated the potential of *C. ternatea* flower extract for several bioactivities. However, most of these studies only used a particular solvent for extraction from the raw material without further purification steps in which there are high chances of various other phytochemicals being present in the crude extract.

The following schematic diagram highlights the dual goal of the study: extraction technique and concentration. The different concentrations of *C. ternatea* extracts are referred to as extract concentrations, and they are commonly divided into low, medium, and high concentrations. Due to the increased availability of bioactive chemicals, previous research has shown that larger concentrations of plant extracts frequently correspond with improved antibacterial activity. Common extraction techniques including methanol, ethanol, and water extraction can produce distinct phytochemical profiles, which may have an immediate impact on the biological activities of the extracts. Preliminary

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

processing of plant extracts was essential for the enrichment and purification of active compounds. Column sorbents such as RP-C18, Toyopearl, Sephadex LH-20, Amberlite XAD-7, and Amberlite XAD-16 have been employed for the separation and enrichment of various phytochemicals. They are known for their unique adsorption properties in which some of these resins have been successfully used for the fractionation and purification of anthocyanins (Jeyaraj et al., 2022).

First, the diagram commenced with the collection of *C. ternatea* samples, focusing on their extraction and testing for antibacterial and cytotoxicity properties. Once the samples are complete, the researchers proceed to the extraction process. Various methods are then applied for the extraction of bioactive compounds from the flower which includes: air drying, oven drying, cutting, soaking/maceration, squeezing, filtration. rotary evaporation and hand picking. Plant extracts contain natural phenolic compounds that are structurally related to a family of water-soluble pigments known as anthocyanins (Suebkhampet A, & Sotthibandhu, P. 2023). Conventional extraction methods usually involve the use of different solvents with heat and/or mixing such as soxhlet extraction, maceration and hydrodistillation which though effective can be costly and require long extraction time (Wen et al. 2018).

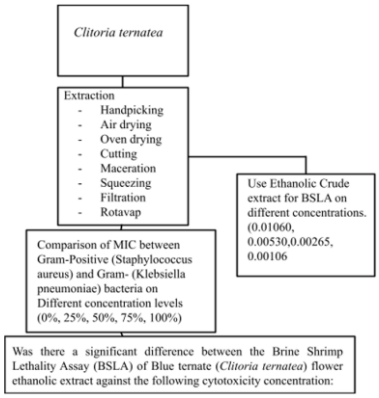
After the extraction process is complete, the study conducted biological testing where the efficacy of the flower extracts was evaluated through two main assays, where the extract was tested against two specific bacterial strains: *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Staphylococcus aureus* is a catalase and coagulase-producing gram-positive organism that frequently occurs in human nasal passages, mucous membranes, or skin of carriers (Qiu et al. 2021). On the other hand *K. pneumoniae* are bacteria that normally live in your intestines and feces. Experts refer to them as Gram-negative, encapsulated, and nonmotile bacteria. They also have a high tendency to become antibiotic resistant (Thottacherry, 2022).

This process is a response to the rising antimicrobial resistance of microbes to conventional drugs (Guimarães et al., 2021). This is a major concern worldwide because even individuals who have never been exposed have been found to carry these resistant strains (Prajna et al., 2022). The antibacterial assay likely involves determining the minimum inhibitory concentration (MIC) of the extracts. The MIC is the lowest concentration of the extract that inhibits visible bacterial growth providing a quantitative measure of the extract's antibacterial potency. *C. ternatea* showed the presence of active components like saponins, tannins, flavonoids, terpenoids, glycosides, amino groups (Pratibhas et al., 2023). Crude ethanolic extract of the fresh *C. ternatea* flowers are used for phytochemical screening, where sterols, terpenoids, flavonoids, Alkaloids, saponins, reducing sugar and tannins shows an Positive result. The presence of various phytochemical constituents contributes to the biological activities of the plant (Torres et al., 2022).

The final phase of the study involves the evaluation of the extracted cytotoxicity using the BSLA (Brine Shrimp Lethality Assay). This assay is based on the ability of the extract to kill laboratory-cultured brine shrimp nauplii, from the genus *Artemia J.L*. (Carballo et al., 2002). This assay was a widely used method for preliminary toxicity screening. Toxicity testing using BSLA was often done for two different purposes. These two lines of research using BSLA offer different perspectives on how the results are interpreted and used. It was often used as a rst-pass tool to screen libraries of extracts or compounds for possible bioactivity, prior to more detailed, more complex bioactivity assays of selected candidates. Through the conceptual framework, the antibacterial and cytotoxic properties of *C. ternatea* flowers were investigated. Finally, the analysis and interpretation of the results and findings integrated the whole conceptual framework of the research.

Figure 1

Schematic Diagram of the Study



BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

4. Significance of the Study

This study was made with the intention of providing knowledge and information regarding the antibacterial efficacy and cytotoxicity of *C. ternatea* flower ethanolic extract. The following may benefit from the substantial information and insights that the study's findings offer:

Urban and Rural Communities. This study will provide knowledge and awareness to the people in this area, as they could benefit from this research by planting *C. ternatea* and having their own source of beneficial plants.

Medical Health Professionals. This research can benefit medical health professionals by serving *C. ternatea* as an alternative medicine to treat a patient.

Pharmaceutical Companies. Pharmaceutical companies can benefit from this research since they can make alternative medicines using the *C. ternatea* flower.

Farmers. This research could benefit farmers by allowing them to sell *C. ternatea* to the pharmaceutical companies.

Department of Health. The Department of Health can utilize this research in a way that they could invent more effective medicines and treatments using the *C. ternatea* plant. They could produce a lot of natural herbal medicines.

Future Researchers. Future Researchers can benefit from this research in a way that they could use this as a review of related literature. They could also add and enhance the research and develop great outcomes in the future.

5. Scope and Limitations

The study addressed the blue ternate (*C. ternatea*) ethanolic extract's Minimum Inhibitory Concentration (MIC) effectiveness against gram-positive and gram-negative bacteria, namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. Moreover, the Brine Shrimp (*Artemia salina*) Lethality Assay (BSLA) assessed the medicinal potential of the plant's bioactive compounds through its cytotoxicity trials. Financial constraints were encountered, which yielded only one trial for MIC. Due to this, the stability of statistics may be questioned, as descriptive analysis was used to translate its results.

Furthermore, MIC was conducted at the Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory, with the supervision of a Microbiological Laboratory Specialist. Meanwhile, the BSLA was conducted at Bacolod Tay Tung High School Biology Laboratory under the supervision of the Laboratory Technician. Both assays were conducted during the School Year 2024-2025. The study did not address the extract's MIC effectiveness against other bacteria or assess the lethality through different procedures.

6. Literature Review

Blue Ternate (Clitoria ternatea) Extract. A study by Yanti et al. (2018) examined the antibacterial, antibiofilm and quorum sensing inhibitory activities of *C. ternatea* anthocyanin against *Streptococcus mutans*. For this experiment, *C. ternatea* was isolated using solvent extraction and underwent broth microdilution method, crystal violet, and *Chromobacterium violaceum* for its quorum sensing inhibition. It was suggested that *C. ternatea* may have therapeutic potentials for prevention and treatment of dental caries (Yanti et.al., 2018).

Jeffrey et al's (2023) research on the antibacterial activity of *C. ternatea* flower against *Streptococcus mutans* and eradicating *S. mutans* biofilm mass further corroborates Yanti's study. The level of inhibition shown by the *C. ternatea* flower extract on the growth of *Streptococcus mutans* was directly proportional to its concentration level, meaning the concentration of the extract, the greater the inhibition produced (Jeffrey et.al., 2023).

The study "Anthocyanin as potential source for antimicrobial activity in *Clitoria ternatea*" by Noraini Mahmad et al. (2018). The researchers revealed the antimicrobial properties of plant extracts that have been shown to be effective in treating bacterial and fungal infections (Mahmad et.al., 2023).

Minimum Inhibitory Concentration. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of silver nanoparticles against *S. aureus* sheds light to its antibiotic-resistant nature from having been exposed to treatments of subjugation in (Parvekar et al. 2020). It was then found that at 99.9%, the bacterial population was killed at the lowest concentration of the antimicrobial agent. Said data presented the findings of bacterial growth inhibition of particular concentrations to be effective.

D'Atanasio et al. used *S. aureus* to study the antibacterial activity of novel DNA type II topoisomerase inhibitors targeting DNA gyrase and topoisomerase IV. The study employed *Escherichia coli* strains, ciprofloxacin, and gel/dilution methods to determine the MBC, while a regression tool was used for LC50 values. MIC was assessed via broth dilution per CLSI guidelines. Strains underwent assays for antibiotic effects and resistance, with mutation

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

frequency in *S. aureus* ATCC 29213 calculated as the CFU/ml ratio on antibiotic plates versus initial colonies (D'Atanasio et al., 2020).

Owuama's study used the Dilution Tube Method to determine MIC and MBC, double diluting the broths containing gentamicin concentrations that inhibit bacterial growth and incubating at 37°C for 18–24 hours. Against *E. coli*, *K. pneumoniae*, and *S. aureus*, MIC tubes showed turbidity, while MBC remained clear. The method proved efficient, eliminating extra stress, time, and costs of agar preparation (Owuama, 2017).

Brine Shrimp Lethality Assay. A research conducted by Waghulde et al. (2019) tested Brine Shrimp Lethality Assay (BSLA) of the Aqueous and ethanolic extracts of Annona reticulata with *Allium fistolisum* and *Brassica oleracea* and correlate cytotoxicity results with known pharmacological activities of the plants. The study's result suggested that these plants may contain bioactive elements, which could explain their pharmacological effects. The findings thus validate the application of these plant species in traditional medicine (Wanghulde et.al., 2018).

Several studies have examined the cytotoxic potential of *C. ternatea* extracts that are prepared using a variety of techniques, the most common of which is BSLA, which is simple and reliable in preliminary toxicity screening. According to (Surya et al. 2024), an ethanolic extract of *C. ternatea* flowers were examined for cytotoxic effects with BSLA17.

A study by Pal et al. (2023) investigated a comparison of the cytotoxic effect of methanolic extracts of leaves and stems of *C. ternatea* by BSLA. They utilized a preliminary, straightforward, high throughput test to assess the cytotoxic behavior of bioactive substances, the brine shrimp lethality bioassay. The results showed that the bioactive compounds present in *C. ternatea* are effectively detected through this assay, reinforcing both the reliability of the methodology and the significance of selecting specific plant parts for extraction18. Their findings confirm that the BSLA test helps screen plant extracts for potential bioactive properties.

7. Methods

Study Design. This research utilized a quantitative research design with a true-experimental approach. A true-experimental design is the most accurate for determining cause-and-effect relationships, requiring a control group, randomization, and manipulated variables (Banaszak & Williams, 2023). Therefore, a true-experimental research approach is suitable for this study, as it employs the Minimum Inhibitory Concentration (MIC) test to evaluate the antibacterial effectiveness of the extract on gram-positive and gram-negative bacteria, and the Brine Shrimp Lethality Assay (BSLA) to measure its cytotoxicity.

Materials. To conduct the study a flower of the *C. ternatea*, 2 plastic bags (1 kilogram), sterilized ziplock bags and a pair of scissors were utilized in picking the flowers. A masking tape and permanent marker for labels. For MIC the materials needed were, *Staphylococcus aureus* and *Klebsiella pneumoniae*. For BSLA, a 5-liter bottle container, aerator, brine shrimp and table salt.

Equipment. The equipment utilized in MIC were Mueller Hinton Broth, Sulfuric Acid, McFarland Standard, Distilled Water Solution, test tubes, UV-visible Spectrophotometer and incubator. For BSLA the equipment used were a rectangular glass jar, measuring cylinder, pasteur pipette, micro tip pipette, test tubes, magnifying glass and *C. ternatea* flower ethanolic extract.

Procedures. In order to conduct this research, the researchers first submitted all necessary forms. After receiving approval, the researchers submitted the plant sample to the Bureau of Plant Industry (BPI) - Bacolod for authentication and verification. The experiment began with the extraction of *C. ternatea*, followed by determining the Minimum Inhibitory Concentration (MIC) using the Broth dilution method. Furthermore, the extract's cytotoxicity was then tested using the Brine Shrimp Lethality Assay (BSLA). The acquired data was thoroughly analyzed and interpreted. Finally, the results on the conducted tests confirmed that *C. ternatea* is a viable alternative for antibiotics.

To analyze and interpret the data, descriptive analysis was used to determine the Minimum Inhibitory Concentration (MIC) across concentrations (0%, 25%, 50%, 75%, and 100%) against *Staphylococcus aureus* (gram-positive) and *Klebsiella pneumoniae* (gram-negative) for Problem Statement 1. Next, the researchers used the statistical treatment, mean and standard deviation, to determine the lethality rate of brine shrimp at each concentration and LC50 to determine *C. ternatea's* cytotoxicity for Problem Statement 2. Lastly, the researchers conducted a One-Way ANOVA to assess differences between Brine Shrimp Lethality Assay (BSLA) results across *C. ternatea* flower ethanolic extract cytotoxicity concentrations for Problem Statement 3.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

8. Results and Discussion

Table 1
Minimum Inhibitory Concentration (MIC) of Blue Ternate (C. ternatea) Flower Ethanolic Extract against S. aureus

Bacterial Sample	Concentration of *C. ternatea* Flower Ethanolic Extract	Minimum Bacterial Concentration (CFU/mL)	Minimum Inhibitory Concentration (CFU/mL)
Staphylococcus aureus	0%	5.6×10^6	Turbid
	25%	7.8×10^6	Turbid
	50%	8.8×10^3	Clear
	75%	3.5×10^2	Clear
	100%	4.8×10^2	Clear

As shown in Table 1, the Minimum Inhibitory Concentration (MIC) readings showed that in sample 1, where the concentration is 0%, the Minimum Bacterial Concentration (MBC) was 5.6×10^6 CFU/mL and its MIC was turbid. It indicated that bacterial growth was present in this concentration. However, at 50% concentration up to 100% concentration, bacterial growth significantly decreased at 8.8×10^3 CFU/mL, and the solution was clear. The MIC of *C. ternatea* extract against *S. aureus* was determined to be at 50%, as this was the lowest concentration that effectively inhibited bacterial growth.

According to a study conducted by Parvekar et al. (2020) supported the findings that after 24 hours of aerobic incubation at 37°C, turbidity was seen in the test tubes containing 0.156 and 0.312 mg/ml of silver nanoparticles, indicating bacterial growth. At concentrations of 0.625, 1.25, 2.5, and 5 mg/ml, no turbidity was observed, indicating inhibition of bacterial growth. These findings validate the MIC and MBC of silver nanoparticles for *S. aureus* was shown to be efficacious at a dilution of 0.625 mg/mL (Parvekar et.al., 2020).

Table 2
Minimum Inhibitory Concentration (MIC) of Blue Ternate (C. ternatea) Flower Ethanolic Extract against K. pneumoniae

Bacterial Sample	Concentration of *C. ternatea* Flower Ethanolic Extract	Minimum Bacterial Concentration (CFU/mL)	Minimum Inhibitory Concentration (CFU/mL)
Klebsiella pneumoniae	0%	6.5×10^6	Translucent
	25%	4.4×10^6	Translucent
	50%	8.7×10^4	Translucent
	75%	7.9×10^3	Translucent
	100%	9.7×10^2	Clear

Table 2 showed that at 0% concentration, the results in Table 2 stated that the bacteria was still at a high amount of bacterial growth at 6.5×10^6 CFU/mL. At 100%, the count has drastically reduced to 9.7×10^2 CFU/mL. At 0% - 75% concentrations, the solution remained translucent, indicating bacterial growth. Moreover, the solution became clear at 100% concentration, signifying effective bacterial inhibition.

According to a study conducted by Owuama (2017), similar findings were achieved with the Dilution Tube Method (DTM) for assessing the MIC and MBC and the new DTM described in the paper. Tubes containing antibiotic concentrations that showed no bacterial growth or turbidity after the first 24 h incubation but showed turbidity after the addition of equal volumes of sterile nutrient broth were said to have minimum inhibitory concentration (Owuama, 2017).

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Table 3
Lethality rate of C. ternatea Flower Ethanolic Extract Against Brine Shrimp (A. salina)

Concentration	Mean	SD
Control Group	4.000	1.732
0.01060 mg/mL	4.333	0.577
0.00530 mg/mL	5.667	3.055
0.00265 mg/mL	5.333	2.082
0.00106 mg/mL	4.000	1.732

The data presented in Table 3 illustrated the effects of different concentrations of *C. ternatea* to assess the cytotoxicity at various doses. The lethality rates showed that the ethanolic extract of *C. ternatea* flowers was non-toxic to *A. salina* across all tested concentrations, ranging from 0.01060 mg/mL to 0.00106 mg/mL. The average lethality levels remained relatively low and comparable to the control group, indicating no substantial toxic effects.

A study by Surya et al. (2024) is aligned with this study since they evaluated the cytotoxic effect of an ethanolic extract of C. *ternatea* flowers using BSLA. The extract demonstrated low toxicity, with an LC50 value of 1,000 µg/ml, indicating minimal cytotoxicity. Similarly the results of this study showed that all tested concentrations of *C. ternatea* extract were non-toxic to *A. salina*, as reflected in the lethality rates, which were comparable to the control group. These findings further support the potential safety of *C. ternatea* flower extract and suggest that it may be a viable candidate for pharmaceutical or medical uses with minimal toxicity concerns (Surya et.al., 2024).

Table 4
Cytotoxicity rate of C. ternatea Flower Ethanolic Extract Against Brine Shrimp (A. salina)

	Concentration	Log_{10} Dose	Empirical Probits	Interpretation
	Control Group	0	0	Non-toxic
	10.60000 ppm	1.03	4.82	Non-toxic
LC_{50}	5.30000 ppm	0.72	5.04	Non-toxic
	2.65000 ppm	0.42	4.86	Non-toxic
	1.06000 ppm	0.03	4.50	Non-toxic

The data in Table 4 indicated that increasing concentrations of the extract resulted in varying levels of cytotoxic effects on the brine shrimp (*A. salina*), as it was stated and measured by the probit values. By using probit analysis in LC_{50}, the researchers identified the cytotoxicity of *C. ternatea* to brine shrimp (*A. salina*). Probit analysis was initially developed to analyze biological and toxicological investigations when the reaction to some stimuli under examination was 'quantal' or 'all-or-none' (Finney, 1952).

A study by Muyiwa et. al. (2019) evaluated the median lethal concentration (LC_{50}) in exposed *Clarias gariepinus* was determined under laboratory circumstances utilizing static non-renewal bioassays throughout a 96-hour exposure with constant aeration. The clinical symptoms detected and its severity varied depending on the dose and exposure period. This study supports these results by demonstrating dose-dependent biological effects of plant-based extracts, reinforcing the importance of controlled concentrations in toxicity and efficacy evaluations (Muyiwa et.al., 2019).

Table 5
Difference Between the Brine Shrimp Lethality Assay of Blue Ternate (C. ternatea) Flower Ethanolic Extract against the following Cytotoxicity Concentrations

Cases	F	df	p	Interpretation
Concentration	0.775	4	0.566	Not Significant

Note: The variance in CFU/mL is equal to 0 after grouping

As shown in Table 5, there was no significant variation in the Brine Shrimp Lethality Assay at varied concentrations. Thus, it indicated that mortality rates at different doses of *C. ternatea* ethanolic extract have minimal impact on brine shrimp lethality compared to the control group. This stated that *C. ternatea* has low toxicity when experimented with brine shrimp (*A. salina*).

A study by Pal et al. (2023) about the cytotoxic effect of leaves and stem extracts of *Clitoria ternatea* by BSLA shows that *C. ternatea* exhibits measurable cytotoxic activity when assessed using the brine shrimp lethality assay. Results showed that the bioactive compounds present in *C. ternatea* are effectively detected through this assay,

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

reinforcing both the reliability of the methodology and the significance of selecting specific plant parts for extraction18. Their findings confirm that the BSLA test helps screen plant extracts for potential bioactive properties (Pal et.al., 2023).

9. Conclusions and Recommendations

Conclusions

The results of this study contributed to a growing body of evidence supporting the efficacy of *C. ternatea* as a potential antibacterial agent. The findings of the Minimum Inhibitory Concentration (MIC) of Blue Ternate (*C. ternatea*) flower ethanolic extract against *Staphylococcus aureus* confirmed that the higher extract concentrations have more potent antibacterial effects. Furthermore, the results of the Minimum Inhibitory Concentration (MIC) of Blue Ternate (*C. ternatea*) flower ethanolic extract against *K. pneumoniae* showed the plant extract's clear dose-dependent antibacterial action. The results indicated that the plant extract has excellent antibacterial activity, especially at higher doses, making it a viable option for alternative antibiotics. Additionally, the researchers determined the lethality rate of *C. ternatea* flower ethanolic extract against Brine Shrimp (*A. Salina*) at different concentrations. The findings further supported the potential safety of *C. ternatea* and suggested that it may be a viable candidate for pharmaceutical or medical uses with minimal toxicity concerns.

Finally, after evaluating the difference in Brine Shrimp Lethality Assay under different concentrations, the results showed no significant difference in mortality rates across various concentrations of *C. ternatea* ethanolic extract. These findings implied that the extract has minimal impact on brine shrimp mortality compared to the control group.

Recommendations

Future studies should conduct this study by having additional trials to further validate and refine the findings. The researchers would also recommend for a stable financial support in order to conduct the experiment in the laboratory. Testing on different bacterial strains is also recommended as it can determine a broader antimicrobial potential. Additionally researchers must explore other BSLA concentrations to assess the variations of toxicity levels. Lastly, a quantitative phytochemical analysis should be conducted to identify and measure the active compounds responsible for the extract's microbial properties.

10. Declaration of Conflict of Interest

Authors have declared that no competing interests exist.

11. Literatures Cited

Banzsak, B., & Williams, Y. (2021). True Experiment Definition & Examples - Video & Lesson Transcript Study – True Experiment: Definition - Studocu. Studocu. https://www.studocu.com/en-au/document/deakin-university/bachelor-of-ar ts-psychology/true-experiment-definition-examples-video-lesson-transcript-study/21199177

D'Atanasio et al. (2020). Antibacterial activity of novel dual bacterial DNA type II topoisomerase inhibitors. PLoS ONE, https://doi.org/10.1371/journal.pone.0228509 15(2), e0228509.

Finney, D. J. (1952). Probit Analysis. Journal of the Institute of Actuaries, 78(3), 388–390. https://doi.org/10.1017/s0020268100052938 Oral

Guimarães et al. (2021). Antibiofilm potential of medicinal plants against Candida spp. biofilms: 10.3390/antibiotics1009114 a review. Antibiotics, 10:1142. doi:

Izah et al. (2024). Antimicrobial Resistance and the Role of Herbal Medicine: Challenges, Opportunities, and Future Prospects. Reference Series in Phytochemistry, 2127–2152. https://doi.org/10.1007/978-3-031-43199-9_71

Jeffrey et al. (2023). Antibacterial effect of telang flower (Clitoria ternatea) extract in eradicating Streptococcus mutans UA 159 Biofilm Mass. Journal of International Dental and Medical Research, 16, 2. http://www.jidmr.com/journal/wp-content/uploads/2023/06/28-D23_2142_J effrey_Indonesia.pdf

Jeyaraj et al. (2021). Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals, 58(6): 2054–2067. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8076379/

Mahmad et al. (2018). Anthocyanin as potential source for antimicrobial activity in Clitoria ternatea L. and Dioscorea alata L. Pigment & Resin Technology, 47(6), 490–495. https://doi.org/10.1108/prt-11-2016-0109

Muyiwa, et al. (2019). Toxicological Evaluation of the Median Lethal Concentration (LC50) of Aqueous Extract of

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Adenium obesum Stem Bark in African Catfish, Clarias gariepinus (Burchell 1822) Juveniles. Toxicology: Open Access, https://doi.org/10.4172/2476-2067.1000140 5(1), 1–8.

Owuama, I. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. African Journal of Microbiology Research, 11(23), 977–980. https://doi.org/10.5897/ajmr2017.8545

Pal, A., Chakraborty, S., Roy, A., & Bala, N. N. (2023). Comparative Study of In vitro Cytotoxic Effect of Leaves and Stems Extracts of Clitoria ternatea by Brine Shrimp Lethality Assay. Journal of Pharmaceutical Research International, 35(21), 47–53. https://doi.org/10.9734/jpri/2023/v35i217411

Parvekar et al. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomaterial Investigations in Dentistry, 7(1), 105–109. https://doi.org/10.1080/26415275.2020.1796674

Philips, J. A., & Blaser, M. J. (2015). Introduction to bacteria and bacterial diseases. In Elsevier eBooks (pp. https://doi.org/10.1016/b978-1-4557-4801-3.00195-8

Qiu, Y., Xu, D., Xia, X., Zhang, K., Aadil, R. M., Batool, Z., & Wang, J. (2021). Five major two components systems of Staphylococcus aureus for adaptation in diverse hostile environment. Microbial Pathogenesis, 159, 105119. https://doi.org/10.1016/j.micpath.2021.105119

Santos-Longhurst, A. (2019, April 3). What You Need to Know About Pathogens and the Spread of Disease. Healthline; Healthline Media. https://www.healthline.com/health/what-is-a-pathogen?fbclid=IwY2xjawJLhYBleHRuA2FlbQIxMAABHVRUes9yHmA0_MbifusLyaoIcmIEGU4MJC-oX EWaAm9-3zj1SpJdX5K_PQ_aem_-08wiBPuCq05W_xJz25rUQ

Septimus, E. J. (2018). Antimicrobial Resistance. Medical Clinics of North America, 102(5), 819–829. https://doi.org/10.1016/j.mcna.2018.04.005

Shirodkar, S. M., Multisona, R. R., & Gramza-Michalowska, A. (2023). The Potential for the Implementation of Pea Flower (Clitoria ternatea) Health Properties in Food Matrix. Applied Sciences, 13(12), 7141. https://doi.org/10.3390/app13127141

Sibanda et al. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. African Journal https://doi.org/10.4314/ajb.v6i25.58241

Soni, J., Sinha, S., & Pandey, R. (2024). Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. Frontiers in Microbiology, 15. https://doi.org/10.3389/fmicb.2024.1370818

Surya, K. G., Shanmugam, R., Behera, A., & Dharmalingam Jothinathan, M. K. (n.d.). Assessment of Antibacterial Properties and Cytotoxic Effect of Ethanolic Extracts of Clitoria ternatea and Camellia sinensis Herbal Formulation Against Clinical Pathogens. Cureus, 16(4), e58287. https://doi.org/10.7759/cureus.58287

Thottacherry, E., & Cortes-Penfield, N. W. (2022). Evidence of clinical impact supports a new petition for Medicare coverage of 18F-FDG-PET/CT in the evaluation of Staphylococcus aureus bacteremia: a focused literature review and call to action. Clin Infect Dis, 75, 1457-61.

Torres, R.C., Parcon, M.R.V. Esmundo, H.J.N., Canillo, D.C.P., Ramil, C.C. (2022). Antioxidant Activity and Phytochemical Constituents of Philippine by Clitoria ternatea Flowers As A Potential Therapeutic Agent Against Infectious Diseases. Issues in Biological Sciences and Pharmaceutical Research Vol.10 (2), pp.12-18. https://doi.org/10.15739/ibspr.22.003

Waghulde, S., Kale, M. K., & Patil, VijayR. (2019). Brine Shrimp Lethality Assay of the Aqueous and Ethanolic Extracts of the Selected Species of Medicinal Plants. Proceedings, https://doi.org/10.3390/ecsoc-23-06703

Wen, C., Zhang, J., Zhang, H., Dzah, C. S., Zandile, M., Duan, Y., Ma, H., & Luo, X. (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops – A review. Ultrasonics Sonochemistry, 48, 538–549. https://doi.org/10.1016/j.ultsonch.2018.07.018

World Health Organization. (2023). Antimicrobial Resistance. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Yanti, Y., Setiawan, T., & Lay, B. W. (2018). Antibacterial, antibiofilm and quorum sensing inhibitory activities of Clitoria ternatea anthocyanin against Streptococcus mutans. International Journal of Infectious Diseases, 73, 143–144. https://doi.org/10.1016/j.ijid.2018.04.3739

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

WOUND HEALING EFFECT OF BAND-AID INFUSED WITH ORANGE (*Citrus x sinensis*) PEEL ETHANOLIC EXTRACT: AN IN VIVO ANALYSIS

Rayliz Anne N. Baldeviso[1], Ana Francessca R. Hermosura[2], Julianna Kim W. Jacildo[3], Matthew Immanuel P. Sayman[4], Philip John G. Tallafer[5], Franchesca Mariz E. Tañeza[6]

[1 2 3 4 5 6] Bacolod Tay Tung High School, Inc., Bacolod City, Philippines

rayliz.baldeviso@btths.edu.ph, ana.hermosura@btths.edu.ph, julianna.jacildo@btths.edu.ph, matthew.sayman@btths.edu.ph, philip.tallafer@btths.edu.ph, franchesca.taneza@btths.edu.ph

Abstract

Natural ethanolic extracts have gained attention due to their antioxidant, anti-inflammatory, and antimicrobial properties, which have potential applications in medicine. However, only a limited number of studies have explored the advantages of natural ethanolic extracts, particularly those derived from citrus fruits and peels like orange (*Citrus x sinensis*), and even fewer have investigated their application in wound healing. This study aimed to determine the wound-healing effects of band-aids infused with orange peel (*Citrus x sinensis*) ethanolic extract on incised wounds. A true experimental quantitative research design was employed. Orange peels were processed into a powder-like substance, subjected to ethanolic extraction, and concentrated through rotary evaporation. Mice (*Mus musculus*) with incised wounds were treated with the infused band-aid, and wound healing was assessed using Percentage Area Reduction (PAR) and Planimetry over seven (7) days. A Mann-Whitney U Test was conducted to compare the PAR between the infused and non-infused band-aid groups. Results indicated that the mice treated with band-aids infused with orange peel ethanolic extract exhibited a significantly faster wound-healing rate than the control group, with a p-value of 0.036. This finding suggested that the extract enhanced the wound recovery process. In conclusion, the study proved that orange peel ethanolic extract significantly affected wound healing compared to standard band-aids, offering a potential natural alternative for wound treatment in medical applications.

Keywords: Ethanol extracts, wound-healing effects, orange (*Citrus x sinensis*), Percentage Area Reduction (PAR), planimetry, and true experimental quantitative research design

1. Background of the Study

Band-aids or adhesive band-aids protect cuts and blisters from bacteria and damage; their only purpose is to cover the wound (Shukla et al., 2020). A study has found band-aids to be less effective and helpful than once thought, and even possibly harmful to human health. The U.S. Environmental Protection Agency-certified lab found that 65% of the bandages contained detectable levels of phthalates, better known as PFAS, the "forever chemicals", posing a threat to human health, specifically bruises and cuts, or also known as incisions (Kuzub, 2025).

An incision is a wound produced by the tearing of soft body tissues. The wound was often contaminated with bacteria and debris from whatever object caused the cut (Arenyeka, 2021). Wounds can be classified as acute, which takes between 7 and 10 days to heal (Criollo et al., 2023). For an incision wound to fully heal, it must undergo four stages: Hemostasis, Inflammation, Proliferation, and Remodeling. Many factors cause improper or impaired incision healing when interfered with one or more stages of this process (Visha et al., 2022). By understanding the basic process of wound healing and conducting a thorough study, the development of effective wound healing products, such as band-aids, can be of great help to the public.

In recent years, the potential of natural crude extracts in wound treatment has gained attention and been included in various studies, particularly those exhibiting antioxidant, anti-inflammatory, and antimicrobial properties, and orange (*Citrus x sinensis*) peel crude extract is one among them (Wong et al., 2020; Miles & Calder, 2021). Citrus fruits, particularly oranges (*C. sinensis*), are known for their high vitamin C content and flavonoids, which can play significant roles in wound healing by promoting collagen synthesis and inflammatory response (Richards, 2021). The extract exhibits a high-value color absorbance, which suggests high antioxidant activity due to its high level of phenols

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

and flavonoids, which can aid in wound healing (Mariano, 2024). Furthermore, integrating natural extracts, such as orange (*Citrus x sinensis*) peel extract, into wound care formulations could enhance healing processes and improve patient outcomes.

Thus, this study aimed to provide a cost-effective and safer alternative to traditional adhesive band-aids with healing properties by integrating orange (*Citrus x sinensis*) peel extract. *In vivo* models, particularly mice, provide valuable insights into the healing process, allowing for the evaluation of potential wound healing properties. Further research could offer a more accessible and cost-effective alternative to traditional band-aids and wound-healing products.

The study results will benefit medical professionals, allowing more accessible and affordable alternatives for treating wound-related injuries. It acted as a stepping stone, providing much-needed answers to fill the gaps in current medical knowledge and further enhancing future research.

2. Statement of the Problem

This study generally aimed to determine the wound-healing effects of the band-aid infused with orange (*Citrus x sinensis*) peel ethanolic extract in incisions.

Specifically, it sought to answer the following questions:

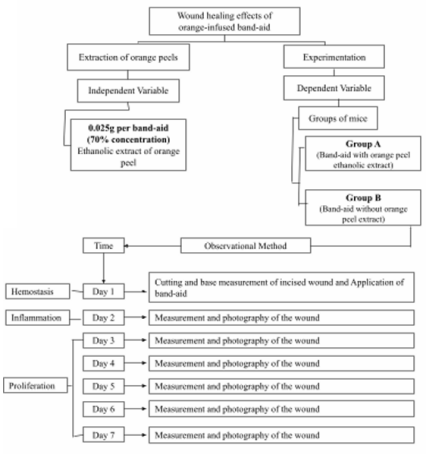
1. What was the average wound area of mice (*Mus musculus*) with Orange (*C. sinensis*) Peel Ethanolic Extract-infused Band-aids and Standard Band-aids on each specific day:
   a) Day 1,
   b) Day 2,
   c) Day 3,
   d) Day 4,
   e) Day 5,
   f) Day 6, and
   g) Day 7?
2. What was the wound healing effect in terms of Percentage Area Reduction (PAR) of band-aids infused with orange (*C. sinensis*) peel ethanolic extract?
3. What was the wound healing effect in terms of Percentage Area Reduction (PAR) when applied using Standard Band-Aids?
4. Was there a significant difference between the average wound healing effect in terms of Percentage Area Reduction (PAR) of Orange (*C. sinensis*) band-aids and standard band-aids at day seven (7)?

3. Framework of the Study

Two studies support the wound healing effects of band-aids infused with orange (*Citrus x sinensis*) extract. Elazabawy et al. (2023) outlined the process of extracting orange peel using a mixture of ethanol and distilled water, followed by filtration and storage at 4°C. The ethanol extraction method yielded the highest results, with 33.6% extraction, and the study identified valuable substances such as fatty hydrocarbon C-H structures and C=O groups in the peel. To remove potentially toxic solvents, a rotary evaporator was used to concentrate the solution without damaging its components. Schultz et al. (2011) discussed the four classical phases of wound healing: hemostasis, inflammation, proliferation, and remodeling, which involve various cells and proteins that support healing. Zlobina et al. (2023) observed that inflammatory genes were upregulated in the early stages of wound healing, followed by proliferation and remodeling phases. These findings are crucial for understanding wound healing and the role of orange peel extract in aiding recovery.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Figure 1
Schematic Diagram of the Study



The researchers conducted an experiment to examine the effects of orange peel extract on wound healing in mice, with the ethanolic solution serving as the independent variable. The experiment was structured around two groups of mice: the control group (Group B), which received a band-aid without the orange extract, and the experimental group (Group A), which received a band-aid infused with the extract. To prepare for the experiment, the researchers determined the right concentration of ingredients in the orange peel solution to adapt it to their experimental needs. The dependent variables in the study were the mouse groups, and their responses were monitored through wound healing over a 7-day period. Each day, researchers measured the width and length of the wounds and took photographs to track healing progress.

4. Significance of the Study

The results of the study will be able to provide significant details and knowledge that can be essential to the following:

Patients. The study's results will benefit the patients by lessening the risks of potential injuries and allowing for quick and easy wound treatment. This will also enable patients to experience an improved and well-balanced quality of life due to the fewer risk of potential wound injuries.

Doctors. The study's results will immensely help doctors with wound-healing-related research in the medical field and allow different alternatives to be accessible to the public. It will act as a stepping stone that will provide answers to fill the gaps in current research.

Healthcare Providers. The study's results will significantly contribute to the different equipment used to treat wound-related injuries and allow for the use of new products.

Pharmaceutical Manufacturers. The study results will provide manufacturers with knowledge in developing more accessible alternatives for wound treatment. Manufacturers could cater to a broader range of consumers who sought affordable yet efficient wound care solutions.

Future Researchers. This research will provide future researchers with materials for conducting a similar study. The study's results will pave the way for future research to deepen the scientific knowledge about the healing effects of band-aids infused with orange (*C. sinensis*) peels.

5. Scope and Limitations

This study used an orange peel ethanolic extract-infused band-aid from orange (*Citrus x sinensis*) peels to assess the wound healing effect. The research was conducted in the Academic Year 2024 - 2025. Moreover, the results cannot be generalized to other citrus fruit peels and other solvents. This extraction of the (*Citrus x sinensis*) orange peel was carried out at Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory. An *in vivo* study was conducted to test the effectiveness of the orange-peel-extract-infused band-aids on mice (*Mus musculus*) for only seven (7) days in the Bacolod Tay Tung High School Biology Laboratory. Male house mice in the weight range of 10-15g

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

and 10 months of age were used for this study. The animals were maintained in clean, polyvinyl cages and fed commercial rat food from a local pet store. Food and water were provided consistently to the animals.

Furthermore, the incision process was done by a Doctor of Medicine with experience in Surgery, and with the supervision of a School Laboratory Technician. The length and width of the wound reduction size will be measured through the Ruler method, using the Percentage Area Reduction (PAR) formula; however, depth was not included. Similarly, this research only looked at the time of wound healing and does not consider the benefits or other potential side effects of using the orange-peel-extract-infused band-aid. Lastly, all processes were carried out according to the stipulations of the Institutional Animal Care and Use Committee (IACUC).

6. Literature Review

Wound Healing. Wound healing is a natural physiological response to tissue injury, involving a complex interplay of cells, cytokines, mediators, and the vascular system (Wallace, 2023). This process occurs in four stages: hemostasis, inflammation, proliferation, and remodeling (Leonard, 2024). Hemostasis begins immediately after injury, where platelets activate and release granules to stop bleeding. The inflammation phase follows, during which enzymes remove bacteria and cellular debris. After two to three days, the proliferation stage starts, promoting tissue regrowth and covering the wound, lasting up to three weeks. Finally, the remodeling phase replaces the wounded area with scar tissue over several weeks (Almadani et al., 2021).

Orange Peel Extract. Orange peel extract is rich in bioactive compounds such as flavonoids, phenolic acids, carotenoids, and limonoids, which offer various medicinal and health benefits (Shrinath et al., 2024). Studies indicate that it possesses strong antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties, making it a valuable natural remedy (Oikeh et al., 2020). The high concentration of vitamin C in orange peel extract aids in collagen synthesis, promoting skin regeneration and immune support (Pelc et al., 2024). Additionally, its antibacterial properties help prevent infections, while its flavonoid content contributes to cardiovascular health and potential anticancer effects (Hosseini et al., 2020). Extracted using solvents like ethanol, orange peel extract is widely utilized in traditional and modern medicine, with applications ranging from skincare to diabetes management and wound treatment (Shehata et al., 2021).

Laboratory Mice. According to Kent Scientific (2022), laboratory mice are widely used in biomedical research due to their genetic similarity to humans, ease of breeding, and short reproductive cycles, making them cost-effective and efficient for experimental studies. They serve as key models for investigating wound healing, inflammation, and tissue regeneration, providing insights into therapeutic interventions such as biomaterials and growth factors (Zlobina et al., 2023). However, their skin differs from human skin due to the presence of the panniculus carnosus, a thin muscular layer that accelerates wound contraction, making direct comparisons to human wound healing challenging (Masson-Meyers et al., 2020). Additionally, mouse skin is thinner than rat skin, leading to a faster healing process, typically within seven days (Mani, 2023). Despite their extensive use, the translation of results from mice to human applications is not always precise due to physiological and pathological differences (Voikar & Gaburro, 2020). Elucidated by the Institutional Animal Care and Use Committee (IACUC) (2022), ethical guidelines for handling and euthanizing laboratory mice are strictly regulated, including humane restraint techniques and approved euthanasia methods such as gaseous carbon dioxide, inhalant anesthetics, and injectable anesthetics to ensure minimal distress.

Band-aid. Band-aids, or adhesive bandages, are essential for protecting minor wounds from bacteria, fungi, and other infectious agents by preventing contamination from dirt and water while also stopping blood loss (Shukla et al., 2024). Studies have shown that hydrogel band-aids accelerate wound healing compared to standard treatments due to their strong mechanical strength, self-recovery properties, and hemostatic activity, making them highly effective in maintaining dressing integrity during the healing process (Chen et al., 2021; Chen et al., 2023). Additionally, plant compounds and extracts with antioxidant and antibacterial properties enhance healing and help prevent wound infections when used in organic band-aids (Khoshkalam et al., 2023). Selecting an appropriate wound dressing is crucial for faster healing, reduced treatment costs, and improved patient well-being (Britto, 2024). According to Buchanan (2025), wounds should be kept moist by applying petroleum jelly when changing the bandage daily, as exposure to air causes cell death and delays healing. A moist environment preserves the wound's natural moisture, preventing scarring and promoting faster recovery.

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

7. Methods

Study Design. This study utilized a quantitative research design, specifically the true experimental research approach, to determine the wound-healing effects of band-aids infused with orange (*Citrus x sinensis*) peel ethanolic extract in an in vivo setting. According to Banaszak (2023), true experimental research is an experiment used to demonstrate or refute a cause-and-effect link between two variables. In this case, the existence of orange (*Citrus x sinensis*) peel ethanolic extract in a band-aid and evaluating its effect on the dependent variable, which is the wound area reduction in mice (*Mus musculus*) over time.

A between-subjects design was also employed, with test subjects separated into two groups, one receiving the orange (*Citrus x sinensis*)-infused band-aid. In contrast, the other received a standard band-aid (control group).

Materials. In the extraction process, the materials were readily prepared: 100g of orange (*Citrus x sinensis*) peels, 100 mL of ethanol (solvent), a 100 mL distilled water for cleaning, a drying oven, a blender, a sieve, a rotary evaporator, a scissors, and an amber bottle.

In conducting the experiment, the following materials were used: 10 pcs of mice, 100 mL of hydrogen peroxide, a scalpel no. 15, a razor, a roll of medical tape, sterile gauze pads, a digital vernier caliper, scissors, sterile gloves, 1 box of face masks, 1.5kg of commercial rat feeds, and 10 plastic containers.

Procedures. This research employed the use of the Ruler Method and Wound Photography as the instruments to collect and support the data. As for the wound contraction, the researchers utilized the Percentage Area Reduction formula. Incisional wounds were cut on the skin and can be categorized as either primary or secondary closure, and mice, rats, rabbits, and pigs can be employed as models. Using a vernier caliper for increased precision, the Ruler method measures the longest length and the widest width of the wound. This often posed a 40% margin of error (Ibraheem et al., 2023); however, by performing three (3) trials per measurement, the results were reduced to 23.1%. The researchers also prepared the homemade band-aids using medical tape and a sterile gauze pad and stored them in a safe and dry area for further use.

Extraction Procedure. First, the oranges were peeled by hand and cut into smaller pieces using scissors. Next, the peels were washed using distilled water and left to air dry in the Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory for 72 hours, isolated to minimize contamination. Once dried, the peels were placed inside the oven at 60-70 °C for 10 days. The dried peels were then crushed in the blender until powdered. The powdered form of the dried peels was then sifted through a sieve to remove chunks and obtain the finer particles. Next, 50g of the powdered peels were macerated in 500 ml of 70% ethanolic extract (v/v water) for 24 hours. Then, the rotary evaporator was used to evaporate the solvent, concentrate the solution, and acquire the crude extract of the peel for 2 hours. Once completed, the crude extracts were transferred to the amber bottles, labeled, and kept in a secure container and stored at 4-10 degrees Celsius in preparation for the experimental procedures.

Testing Procedure. Before the testing procedure, the researchers obtained ethical animal use and care approvals, gathered approval, and prepared essential equipment like sterile gloves, masks, and laboratory gowns. They prepared the mouse subjects, dividing them into A and B groups of five mice each, each experiencing different conditions. The mice were isolated, and their containers were labeled with animal IDs. All materials were sterilized with Hydrogen Peroxide (H_2O_2) to prevent contamination. The medical professional used chloroform inhalation to anesthetize the mice, then shaved and made incisions on their thighs using a sterilized scalpel, ensuring consistent cut sizes. The initial wound area was measured using a digital vernier caliper, repeated for three trials. The researchers applied homemade orange-peel-extract-infused band-aids to Group A and standard ones to Group B, securing them around the hind legs. Over seven days, they observed and measured wound areas daily, replacing band-aids, maintaining clean living conditions, and consistently feeding the mice. After gathering data, they calculated the percentage of wound area reduction interpreted by the school's statistician and discussed by the researchers. Once the experiment concluded, the mice were euthanized using overdosing of chloroform ($CHCl_3$) following IACUC protocol, and disposed of properly by the School Safety Control Officer.

Data Analysis Procedure. To address the research problems, each variable was determined through different processes with multiple limitations. The goal was to determine the average effectiveness of band-aids in daily wound healing using the mean across three trials for Problem 1. For Problems 2 and 3, the researchers aimed to calculate the average wound healing effect of both orange (*Citrus x sinensis*) peel ethanolic extract-infused and standard band-aids by Day 7 using the Percentage Area Reduction (PAR) formula. Finally, for Problem 4, they sought to compare the difference in wound healing effects between orange-peel-extract-infused and standard band-aids using the Mann-Whitney U-Test.

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

8. Results and Discussion

Table 1
Average wound area (in cm²) of mice (Mus musculus) with orange-peel-extract-infused band-aids on each specific day

Day	A – 1	A – 2	A – 3	A – 4	A – 5
1	0.063365 cm^2	0.061824 cm^2	0.079677 cm^2	0.071077 cm^2	0.093632 cm^2
2	0.053756 cm^2	0.032040 cm^2	0.064512 cm^2	0.051624 cm^2	0.036453 cm^2
3	0.030248 cm^2	0.027462 cm^2	0.047530 cm^2	0.021128 cm^2	0.015695 cm^2
4	0.017577 cm^2	0.015048 cm^2	0.028248 cm^2	0.012027 cm^2	0.010430 cm^2
5	0.012243 cm^2	0.009472 cm^2	0.023542 cm^2	0.004173 cm^2	0.006670 cm^2
6	0.006960 cm^2	0.002369 cm^2	0.009546 cm^2	0.002759 cm^2	0.002373 cm^2
7	0.009381 cm^2	0.008618 cm^2	0.003264 cm^2	0.000736 cm^2	0.001748 cm^2

Table 1 presents the average area of each mouse in Group A, the experimental group that received band-aids infused with orange peel (*Citrus x sinensis*) extract. Utilizing the Ruler method, where the longest length and the widest width were measured to get the area (Swift, 2020), and three trials were conducted to minimize errors.

Figure 2
Series of Wound Photography of the Group A (Experimental Group) Wound Area Daily

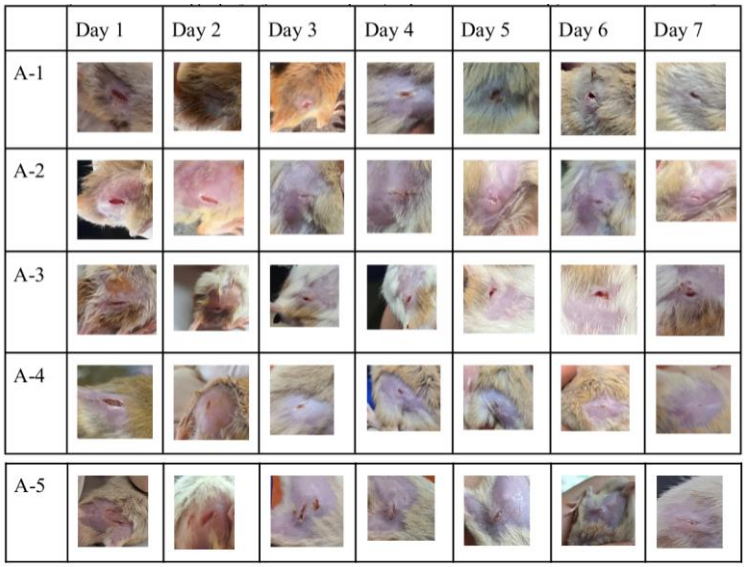


Table 2
Average wound area (in cm²) of mice (Mus musculus) with band-aids without orange peel extract on each specific day

Day	B – 1	B – 2	B – 3	B – 4	B – 5
1	0.121094 cm^2	0.189040 cm^2	0.201231 cm^2	0.124914 cm^2	0.141795 cm^2
2	0.104550 cm^2	0.158457 cm^2	0.160080 cm^2	0.119140 cm^2	0.135519 cm^2
3	0.081620 cm^2	0.145700 cm^2	0.118372 cm^2	0.079730 cm^2	0.118312 cm^2
4	0.068715 cm^2	0.128316 cm^2	0.077792 cm^2	0.073962 cm^2	0.078540 cm^2
5	0.062830 cm^2	0.066993 cm^2	0.064128 cm^2	0.047628 cm^2	0.056610 cm^2
6	0.026220 cm^2	0.035378 cm^2	0.025454 cm^2	0.024624 cm^2	0.064350 cm^2
7	0.024840 cm^2	0.027795 cm^2	0.016048 cm^2	0.018848 cm^2	0.040300 cm^2

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Table 2 presents the quantitative measurements of the average wound area for Group B, the control group that used the standard band-aids. The measurement method is exactly the same as that of Group A.

Figure 3

Series of Wound Photography of the Group B (control group) Wound Area Daily

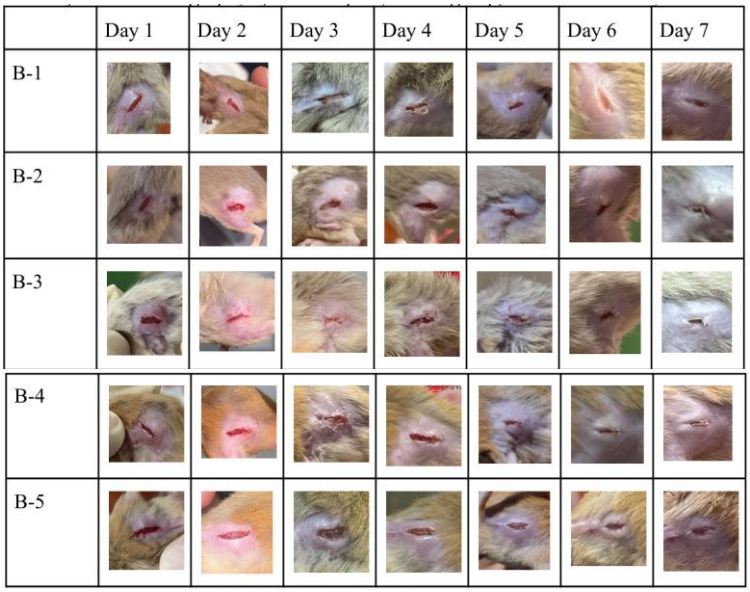


Figure 3 is the wound series photography of group B over the course of 7 days.

Table 3

Percentage Area Reduction (PAR) of Band-Aids Infused with Orange (C. sinensis) Peel Ethanolic Extract

Animal ID	Initial Wound Area	Final Wound Area	PAR
A – 1	0.063365 cm^2	0.009380 cm^2	85.20%
A – 2	0.061824 cm^2	0.008618 cm^2	86.06%
A – 3	0.079677 cm^2	0.003264 cm^2	95.90%
A – 4	0.071077 cm^2	0.000736 cm^2	98.96%
A – 5	0.093632 cm^2	0.001748 cm^2	98.13%

Table 3 presents the Percentage Area Reduction (PAR) of Group A from Day 1 to Day 7. Group A's mice had the PAR of 85.2% up to 98.96%, presenting a high reduction.

Table 4

Percentage Area Reduction (PAR) of Band-Aids Without Orange (C. sinensis) Peel Ethanolic Extract

Animal ID	Initial Wound Area	Final Wound Area	PAR
B – 1	0.121094 cm^2	0.024840 cm^2	79.00%
B – 2	0.189040 cm^2	0.027795 cm^2	85.00%
B – 3	0.201231 cm^2	0.016048 cm^2	92.00%
B – 4	0.124914 cm^2	0.018848 cm^2	85.00%
B – 5	0.141795 cm^2	0.040300 cm^2	72.00%

Table 4 presents the Percentage Area Reduction (PAR) of Group B from Day 1 to Day 7. Group B's mice had the PAR of 72% up to 92%, presenting varied reduction.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Table 5
Average Wound Healing Effect in terms of Percentage Area Reduction (PAR) of Orange Peel (C. sinensis) Ethanolic Extract-Infused Band-Aids and Standard Band-Aids

Day	Group A	Group B
1	0.073915 cm^2	0.1556148 cm^2
2	0.047677 cm^2	0.1355492 cm^2
3	0.0284126 cm^2	0.1087468 cm^2
4	0.0675124 cm^2	0.0854650 cm^2
5	0.0112200 cm^2	0.0596378 cm^2
6	0.0048014 cm^2	0.0352048 cm^2
7	0.0047494 cm^2	0.0255662 cm^2

Table 5 showed the average area of the experimental and control groups over the course of seven (7) days.

Table 6
Difference Between the Average Wound Healing Effect in terms of Percentage Area Reduction (PAR) of Orange Peel (Citrus x sinensis) Ethanolic Extract - Infused Band-Aids and Standard Band-Aids

Percentage Area Reduction (PAR)	U	*p*	Interpretation
	23.000	0.036	Significant

Table 6, the concluding data, offered a final interpretation of the study's results. A Shapiro-Wilk test conducted by the school statistician indicated a *p*-value of 0.460, suggesting that the data deviated from normality. Therefore, a non-parametric test, such as Mann-Whitney, was employed. With the U-test value of 23.000, the Mann-Whitney U-test yielded a *p*-value of 0.036, indicating a statistically significant difference in Percentage Area Reduction (PAR) between the infused band-aids and the standard band-aids.

9. Conclusions and Recommendations

Conclusions

The orange (*Citrus x sinensis*) peel-infused band-aid group showed a significantly higher Percentage Area Reduction (PAR) compared to the control, indicating enhanced wound healing. The statistical difference implies that the healing did not happen by chance; rather, there was an actual wound-healing effect using the orange peel extract. This finding was further supported by visual evidence from wound photography. This aligns with studies by Dahmani et al. (2020), Cherukuri et. al (2023), and Sovia et al. (2021), where different and similar species of oranges all have exhibited a positive wound healing effect.

Recommendations

Based on the findings of the study presented, the following recommendations for the best course of action are given by the researchers to the significant groups of people:

Patients. This study will be helpful to the patients who require care. The findings of this research are informative to them as they can use them as a basis and a potential substitute to take care of their wounds.

Doctors. Doctors can prescribe the use of orange peels and their wound-healing properties to patients. Oranges (*Citrus x sinensis*) can widen the potential uses for medicinal and therapeutic purposes.

Healthcare Providers. The study's results may benefit healthcare providers by considering the potential health benefits that orange peels can contribute to wound healing.

Pharmaceutical Manufacturers. Pharmaceutical manufacturers should consider developing orange peel extract into band-aids to aid wound healing due to the different properties and available bioactive compounds.

Future Researchers. Future researchers should replicate this study to validate results, test orange peel extract on human-like skin models, consider gender-specific responses, and explore varying dosages for comparative analysis.

10. Declaration of Conflict of Interest

Authors have declared that no competing interests exist.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

11. Literatures Cited

Almadani, Y., Vorstenbosch, J., Davison, P., & Murphy, A. (2021). Wound Healing: A Comprehensive Review. Seminars in Plastic Surgery. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8432991/

Arenyeka, U. V. (2021). The Contributions of Sports Science in the Prevention and Rehabilitation of Injuries Sustained During Sports and Physical Activity. Nigeria Academic Forum, Vol. 28 No. 1. ISSN: 1596-3306. https://www.globalacademicgroup.com/journals/the%20nigerian%20academic%20forum/V28N1P28_2021_NAF.pdf

Banaszak, B. (2023). True Experiment | Definition, Components & Examples. Psychology Courses. Study.com. Retrieved from https://study.com/academy/lesson/true-experiment-definition-examples.html#:~:text=A%20true%20experimental%20method%20is,and%20a%20researcher%2Dm anipulated%20variable.

Britto, E. J., et. al. (2024). Wound Dressings. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK470199/

Buchanan, A. M., (2025). Do Wounds Need Air to Heal? UPMC HealthBeat. https://share.upmc.com/2021/10/do-wounds-need-air-to-heal/

Calder, P. C. & Miles, E. A. (2021). Effects of Citrus Fruit Juices and Their Bioactive Components on Inflammation and Immunity: A Narrative Review. Front. Immunol. 12:712608. doi: 10.3389/fimmu.2021.712608

Chan, E. W. C., Wong, S. K., & Foster, S. A. (2020). Drying of food and herbal materials: A review. *Journal of Food Processing and Preservation*, 44(5), e14402. https://pmc.ncbi.nlm.nih.gov/articles/PMC7168226/

Chen, X., et. al. (2021). Bioactive skin-mimicking hydrogel band-aids for diabetic wound healing and infectious skin incision treatment. Bioactive Materials, 6(11), 3962–3975. https://doi.org/10.1016/j.bioactmat.2021.04.007 Chen, Y., et. al. (2023). Light-controlled crosslinked multifunctional "Band-Aids" as dual-stage wound dressing for dynamic wound closure. Collagen and Leather, 6(1). https://doi.org/10.1186/s42825-024-00167-5

Cherukuri, S., et. al. (2023). Evaluation of Cutaneous Wound Healing Activity of Citrus aurantium Fruit Peel Extract-based Ointment in Albino Rats. Research Journal of Pharmacy and Technology, https://doi.org/10.52711/0974-360x.2023.00046 250–254.

Criollo-Mendoza, M. S., Contreras-Angulo, L. A., Leyva-Lopez, N., Gutierrez-Grijalva, E. P., Jiménez-Ortega, L. A., & Heredia, B. J. (2022). Wound Healing Properties of Natural Products: Mechanisms of Action. *Novel Natural Compounds as Wound Healing Agents*, 2(28), 598. https://doi.org/10.3390/molecules28020598

Dahmani, S., Chabir, R., Errachidi, F., Berrada, W., Lansari, H., Benidir, M., Ghadraoui, L. E., & Bour, A. (2020). Evaluation of in vivo wound healing activity of Moroccan Citrus reticulata peel extract. *Clinical Phytoscience* volume 6, Article number: 78. Springer Open. Available from https://clinphytoscience.springeropen.com/articles/10.1186/s40816-020-00222-8

Elastoplast. (n.d). Different Types of Wounds | Open & Closed Wounds. www.elastoplast.co.uk. https://www.elastoplast.co.uk/did-you-know/health-and-protection/types-of-wound

Hosseini, S. M., Hoseinifar, S. H., Mazandarani, M., Paknejad, H., Doan, H. V., & El-Haroun, E. R. (2020). The potential benefits of orange peels derived pectin on serum and skin mucus immune parameters, antioxidant defence and growth performance in common carp (Cyprinus carpio). Fish & Shellfish Immunology, 103, 17-22. https://doi.org/10.1016/j.fsi.2020.04.019

Ibraheem, W., Bhati, A., Hakami, N., Alshehri, A., Wadani, M., & Ageeli, F. (2023). Comparison of digital planimetry and ruler methods for the measurement of extraction socket wounds. *Medicina*, 59(1), 135. https://doi.org/10.3390/medicina59010135 Medicina, 59(1), 135.

Institutional Animal Care and Use Committee (2020) Basic Mouse Handling and Technique Guide. Division of Comparative Medicine (DCM). Available from https://research.unc.edu/wp-content/uploads/2020/12/mouse-handling-and-techniques.pdf?fbclid=IwZXh0bgNhZW0CMTEAAR2hApZA0N3CUqgYCHHbZHB0HF4aQGAonxFTlwEozsdXtKOYjdi-lLdG8Hc_aem_WrEwSBy4sj7HfCbZtHLwJw

Karunagaran, M., & Visha, M. (2022). A review on wound healing. International Journal of Clinicopathological Correlation, 3(2), https://www.editorialmanager.in/index.php/ijcpc/article/view/404 50.

Kent Scientific (2022) Why are rats used in research? Kent Scientific: Mouse & Rat Laboratory Research Equipment. https://www.kentscientific.com/blog/why-rats-used-research/

Khoshkalam, B., et. al. (2023). An Evaluation of Anti-Microbial Properties of Gelatin and Collagen Films Enriched by Aloe Vera and Henna to Build an Organic Band-Aid. Herbal Medicines https://doi.org/10.22087/hmj.v8i1.1000 Journal (Herb Med J), 8(1).

Kuzub, A. (2024). The report says band-aids contain PFAS, the 'forever chemicals.' many small exposures can add up

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

to a big exposure, environmental expert warns. Northeastern Global https://news.northeastern.edu/2024/04/09/band-aid-pfas-forever-chemicals/ News.

Leonard, C. (2024) Four stages of wound healing: Vohra wound physicians. Vohra Wound Care. https://cert.vohrawoundcare.com/the-four-stages-of-wound-healing-an-updated-overview-for-clinicians/

Mani, A. (2023). From mouse to human. *eLife*, 12. https://doi.org/10.7554/elife.94382

Mariano, F. I. (2024). Antioxidant and Anti-Arthritic Activity of Peels from Citrus Fruits. *Science Asia Review*, 9(1), 1–1. https://ejournals.ph/article.php?id=25427

Masson-Meyers, D. S., Andrade, T. A. M., Caetano, G. F., Guimaraes, F. R., Leite, M. N., Leite, S. N., & Frade, M. A. C. (2020). Experimental models and methods for cutaneous wound healing assessment. *International journal of experimental pathology*, 101(1-2), 21–37. https://doi.org/10.1111/iep.12346

Oikeh, E. I., Oviasogie, F. E., & Omoregie, E. S. (2020). Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis (L.) Osbeck (sweet Orange) peels. *Clinical Phytoscience*, 6(1). https://doi.org/10.1186/s40816-020-00193-w

Pelc, C. (2024). Could orange peels help improve heart health? medicalnewstoday.com. https://www.medicalnewstoday.com/articles/orange-peels-extract-help-improve-h eart-health

Richards, L. (2021). What are the health benefits of mandarin oranges? https://www.medicalnewstoday.com/articles/mandarin-orange/

Shrinath, V.J., et al. (2023). Antioxidant Properties of Orange Peel and Their Implications for Health: A Comprehensive Review. *J Food Chem Nanotechnol* 9(S1): S546-S553. https://foodchemistryjournal.com/2023/11/15/antioxidant-properties-of-orange-pe el-and-their-implications-for-health-a-comprehensive-review/

Shukla, D., et al. (2022). A Review on Use of Herbal Plant Sources in Adhesive Bandages for Wound Healing Process. *Indo-American Journal of Pharmaceutical Research*. https://iajpr.com/iajprfiles/uploaddir/220107_DOI.pdf

Sovia, E., Harjono, R., Nurjatin, R., Asmara, W., & Rohmah, A. (2021). Effect of Sweet Orange and Pomegranate Peel Extract Combination on in Vivo Antidiabetic and Wound Healing Activity. https://www.atlantis-press.com/proceedings/asmc-21/125959087

Shehata, M. G., Awad, T. S., Asker, D., El Sohaimy, S. A., Abd El-Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science*, 4, 326-335. https://doi.org/10.1016/j.crfs.2021.05.001

Swift. (2020). Wound Measurement, Assessment and Documentation. Swift. https://swiftmedical.com/wound-measurement-assessment-and-documentation/

Voikar, V. & Gaburro, S. (2020). Three pillars of automated home-cage Phenotyping of mice: Novel findings, refinement, and reproducibility based on literature and experience. *Frontiers*. https://doi.org/10.3389/fnbeh.2020.575434

Wallace, H. A., Zito, P. M., & Basehore, B. M. (2023). Wound Healing Phases. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK470443/

Zlobina, K., Malekos, E., Chen, H., & Gomez M. (2023) Robust classification of wound healing stages in both mice and humans for acute and burn wounds based on transcriptomic data. BMC Bioinformatics. Part of Springer Nature. Retrieved from https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-023-05295-z#:~:text=Wound%20healing%20is%20a%20dynamic,remodeling%20%5B1%2 C%202%5D

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF SPLIT GILL MUSHROOMS (*Schizophyllum commune*) CRUDE EXTRACT AGAINST *Staphylococcus aureus*

Jeremiah U. Barredo[1], April Anne E. Dealagdon[2], Katherine Joy F. Ganchoon[3], Neil Jahrud C. Pulmones[4], Kate Ashley U. Torrejon[5], Irish Nicole F. Tumayan[6]

[1 2 3 4 5 6] Bacolod Tay Tung High School, Inc., Bacolod City, Philippines

jeremiah.barredo@btths.edu.ph, april.dealagdon@btths.edu.ph, katherine.ganchoon@btths.edu.ph, neil.pulmones@btths.edu.ph
kate.torrejon@btths.edu.ph, irish.tumayan@btths.edu.ph

Abstract

Split Gill mushrooms (*S. commune*) are among the most common wild mushroom species across the globe and in the Philippines, commonly utilized by indigenous communities for nutraceutical purposes due in part to its supposed beneficial properties and possible potential to be used as a natural alternative for medicine. This study aimed to investigate the phytochemical content of *S. commune* and its antimicrobial properties against the gram-positive bacterium *Staphylococcus aureus* using three crude extracts. A quantitative research design with an experimental approach was employed for the study, and a series of laboratory experiments were conducted: phytochemical screening and antimicrobial assay utilizing the Kirby-Bauer Disk Diffusion test. The results of the experiments were analyzed using descriptive statistics in addition to Mann-Whitney tests, Kruskal-Wallis test, and Dunn's test for data comparisons. The phytochemical screening identified the presence of alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids in S. commune. Antimicrobial testing revealed that *S. aureus* was resistant towards the different extracts, with ethanol exhibiting the highest inhibitory activity. Statistical analysis revealed a significant difference between the inhibition rates of the aqueous, ethanol, and isopropanol crude extracts at 80% concentration levels. Based on the results, *S. commune* contains a number of phytochemicals that will be essential for its biological activities. To gain the highest inhibition rate against *Staphylococcus aureus* using *S. commune*, it should be extracted using ethanol as a solvent.

Keywords: Mycology, Split Gill mushroom (*Schizophyllum commune*), bioactive compounds, phytochemical screening, antimicrobial assay, Kirby-Bauer disk diffusion test, *Staphylococcus aureus*, quantitative research

1. Background of the Study

Fungi are highly diverse groups of eukaryotic microorganisms (Morton, 2021). They have been utilized and cultivated for various purposes since the early traces of civilization: consumption, social, religious, and medicinal. Countries such as China hold records of the usage of fungi for traditional medicine. One such example of the fungi utilized commonly for the properties above is Split Gill mushrooms (Schizophyllum commune) (Prabsangob & Sittiketgorn, 2023). *S. commune* is a fungus present in every continent. It commonly grows on the dead branches of deciduous trees (Missouri Department of Conservation, n.d). It is among the most frequently found wild mushroom species in the Philippines, commonly utilized by Indigenous communities for nutraceutical purposes (De Leon et al., 2021; Dulay et al., 2022). *S. commune* has been studied before and has exhibited antioxidant, anti-inflammatory, and antimicrobial properties (Razak et al., 2024). These properties are possible due to fungi being a potent source of secondary metabolites such as phytochemicals. (Dutta et al. 2023). Phytochemicals are secondary metabolites in plants that stimulate biological activities (e.g. antioxidant and antimicrobial activity) (Gemechu, 2020). This is why phytochemicals are among the most researched topics in studies, especially those within related fields. The antioxidant and antimicrobial properties presented by phytochemicals within fungi hold the potential to create products or substances that can combat microorganisms such as viruses and bacteria. Bacteria can be classified into two via Gram stain, wherein a culture of bacteria is stained using a crystal violet or methylene blue dye and checked whether or not the bacteria retain the dye after solvent treatment (Tripathi & Sapra, 2023).

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

A study conducted by Khardziani et al. (2020) tested various mushrooms found within the mountainous and forested regions of the nation for possible antimicrobial activities. Of the chosen thirty mushrooms, *S. commune* showed the highest antimicrobial activity against selected gram-positive bacteria, having the widest inhibition zones. A study conducted by Al-Azad & Ping (2022) tested two species of mushroom, one of those being *S. commune*, for antioxidant properties and antimicrobial activities. The samples were turned into aqueous, 50% ethanol, methanol, and acetone crude extracts. The results showed that *S. commune* possesses antimicrobial activity, specifically, antibacterial. A study conducted by Acanto & Cuaderes (2021) in Negros Occidental showed that crude extracts of S. commune can inhibit gram-positive bacteria due to the various phytochemical compounds present that are associated with antimicrobial activity, such as alkaloids, flavonoids, and terpenoids.

While research exists on the antimicrobial properties of *S. commune*, the addition of more literature is necessary, covering the use of other extracts and at various concentration levels.

2. Statement of the Problem

This study aimed to investigate whether or not Split Gill mushrooms (*S. commune*) contain antimicrobial properties and observe what these properties did against the selected gram-positive bacterium *Staphylococcus aureus*.

Specifically, it sought to answer the following questions:

1. What phytochemical compounds are present in *S. commune*?
2. What was the inhibition rate of *S. commune* against *Staphylococcus aureus* in the following concentrations:
   a) 80% concentration of aqueous crude extract of *S. commune*,
   b) 80% concentration of ethanol crude extract of *S. commune*, and
   c) 80% concentration of isopropanol crude extract of *S. commune*?
3. Is there a significant difference between the inhibition rates of *S. commune* against *Staphylococcus aureus* in the following concentrations:
   a) 80% concentration of aqueous crude extract of *S. commune*,
   b) 80% concentration of ethanol crude extract of *S. commune*, and
   c) 80% concentration of isopropanol crude extract of *S. commune*?

3. Framework of the Study

The foundations of this study on the Phytochemical and Antimicrobial Screening of Split Gill Mushrooms (*Schizophyllum commune*) Crude Extract Against *Staphylococcus aureus* were supported by the concepts of Behavioral Ecology and Fungal Competition, Biofilm Inhibition, and the research conducted by various sources confirming theories on the efficacy of Traditional Medicine and the Bioactivity of Medicinal Mushrooms.

Behavioral Ecology and Fungal Competition. With the exception of the Antarctic continent, Split Gill mushrooms or *Schizophyllum commune* can be found growing on the bark of trees all over the world and are capable of degrading polysaccharides present in the cell walls of plants (Barnes et al., 2023). As a wood-decaying fungus, it may have evolved antimicrobial compounds in response to threats from other organisms.

An experimental study conducted by Krause et al. (2020) observed three main responses when *S. commune* was grown with other fungi, namely deadlock, replacement, and induction. Deadlock refers to a mutual inhibition between two competing species where neither is able to outgrow the other (Brglez et al., 2020) and occurred during the interaction between *Schizophyllum commune* and *Flammulina velutipes*. Replacement occurred with *Serpula lacrymans* and induction with *Ganoderma lucidum* wherein *S. commune* growth was promoted.

Biofilm Inhibition. Biofilms are defined as a community of microorganisms embedded in an extracellular matrix composed of polymeric substances (Mello et al., 2019). They serve as a defensive formation against a host's immune system, often helping various harmful microorganisms to grow and establish chronic infections (Costerton et al., 1999). Pathogenic microbes are also present in biofilms, making treatment difficult due to their strong resistance to antimicrobials (De La Fuente-Núñez et al., 2013).

However, a recent study on *Schizophyllum commune* showed that the fungus possessed good antibacterial and anti-biofilm activity against pathogenic bacteria such as *Staphylococcus aureus*, Klebsiella pneumoniae, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Vibrio cholerae* (Sharma et al., 2024). This suggests that *S. commune* has antimicrobial potential.

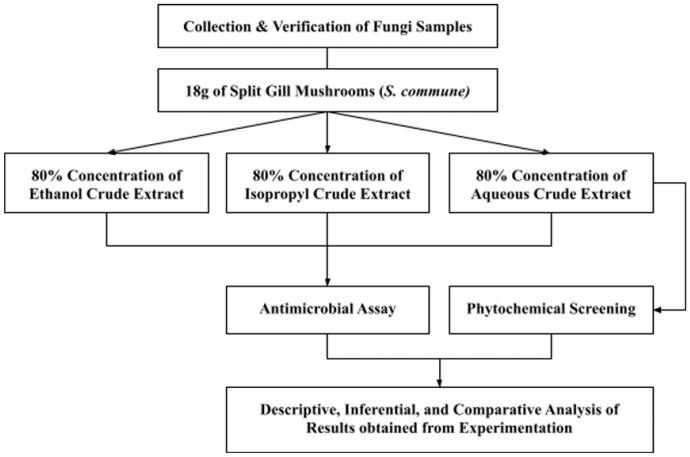
Theories in Traditional Medicine and Bioactivity of Medicinal Mushrooms. Humans have depended on natural resources for millennia in order to satisfy essential needs such as food, water, shelter, clothing, and tools. Throughout history, surrounding environments have been explored to enhance quality of life. This exploration involved identifying

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

various organisms with potential benefits, particularly in health. Traditional medicine utilized natural products such as plants, and modern research continues to investigate different organisms with potential medicinal properties.

Mushrooms, in particular, have been a component of traditional medicine for centuries, with studies confirming their bioactivity, showing that mushroom extracts from various species exhibited antidiabetic, antihypercholesterolemic, antihypertensive, anti-neurodegenerative, antioxidative, antitumor, and antimicrobial activities (Luan et al., 2022; Łysakowska et al., 2023; Shevchuk et al., 2023; Stajić et al., 2023).

Figure 1
Schematic Diagram of the Study



In order to further understand the phytochemical and antimicrobial screening of Split Gill mushrooms (*Schizophyllum commune*) crude extracts against *Staphylococcus aureus*, a research framework was developed to serve as a basis and guide. Samples of *S. commune* were collected from local forests within Negros Occidental and verified by experts in the field. The samples were then delivered to the laboratory of Negros Prawn Producers Cooperative and made into crude extracts, specifically, aqueous, ethanol, and isopropanol to prepare for the testing phase. The gram-positive bacterium *Staphylococcus aureus* was acquired from biological institutions in Bacolod City. The processed samples of fungi and bacterium were then used to perform agar plate diffusion, an assay used to test the susceptibility of bacteria to antibiotics. This involved the spreading of bacteria on an agar plate, application of an antibiotic, and observing if a zone of inhibition occurred, which would indicate whether or not bacterial growth has been inhibited. One of the plates was also subjected to phytochemical screening prior to the agar diffusion test to determine the presence of any bioactive compounds within the sample of *Schizophyllum commune*. The results of the experiment were analyzed, evaluated, and interpreted by the researchers to assess, discuss, and conclude the findings.

4. Significance of the Study

The results of the study has provided significant details and knowledge which could be essential to the following:

Department of Health (DOH). The Department of Health is responsible for ensuring public health and safety. This study's findings could inform the DOH about potential new natural antimicrobial agents derived from *S. commune*. If effective against gram-positive bacteria, these compounds could be integrated into public health strategies to combat infections, especially in the face of rising antibiotic resistance. Therefore, potentially reducing healthcare costs associated with antibiotic treatments.

Department of Environment and Natural Resources (DENR). The DENR is tasked with protecting the country's natural resources and promoting sustainable practices. This study's exploration of *S. commune* not only highlights the ecological importance of fungi but also underscores their potential role in bioprospecting for antimicrobial substances. By understanding the antimicrobial properties of native mushrooms, the DENR can advocate for the conservation of biodiversity and promote sustainable harvesting practices. Thus, ensuring these valuable resources remain available for future research and application.

Healthcare Professionals. Healthcare professionals are responsible for diagnosing, treating, and promoting health among patients. The findings of this study will equip them with information on the potential antimicrobial effects of

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

S. commune against selected gram-positive bacteria, which could lead to new treatment options or adjunct therapies in clinical settings. Thus, may enhance their ability to combat bacterial infections effectively and improve patient outcomes.

Pharmaceutical Industries. The pharmaceutical industries are responsible for developing medications and therapies aimed at improving health outcomes for patients. Insights from this study can guide the development of new natural products or supplements that target specific bacterial infections. Hence, it could lead to innovative solutions in pharmaceutical applications and contribute to the development of alternative antimicrobial therapies.

Indigenous People. Indigenous communities often possess extensive traditional knowledge regarding the use of local flora, including mushrooms for medicinal purposes. The study could provide scientific validation that can reinforce these traditional practices and offers empirical support for indigenous healing methods, affirming their cultural significance. If *S. commune* is proven to be an effective natural antimicrobial agent, it can serve as a sustainable alternative to synthetic antibiotics, aligning with indigenous philosophies that emphasize harmony with nature and reducing reliance on chemical treatments. Additionally, the findings may foster a greater appreciation for local biodiversity among indigenous communities, encouraging conservation efforts and sustainable use of natural resources; empowering indigenous people by equipping them with evidence to advocate for their health practices within broader healthcare discussions. Thus, this study could validate indigenous beliefs and promote sustainable health practices, economic development, and biodiversity appreciation, enriching both cultural identity and scientific understanding.

Environmentalists. Environmentalists advocate for preserving ecosystems and promoting sustainable use of natural resources. The study on *S. commune* aligns with their goals by emphasizing fungi's role in providing natural antimicrobial solutions that can reduce reliance on synthetic chemicals in healthcare and agriculture. The study may encourage further exploration into other local fungal species, fostering a greater appreciation for biodiversity and its potential benefits. Additionally, if *S. commune* proves effective as a natural antimicrobial agent, it could lead to decreased environmental impact from chemical pollutants associated with conventional antibiotics.

Future Researchers. This study will benefit future researchers by providing further knowledge and ideas that serve as a foundation for further research. Furthermore, this will act as a reference for future inquisitions.

5. Scope and Limitations

This study focused on the phytochemical analysis and antimicrobial screening of crude extracts from Split Gill mushrooms (*S. commune*) to identify and quantify key bioactive compounds (i.e., phytochemicals). It assessed the antimicrobial efficacy of *S. commune* via aqueous, ethanol, and isopropanol crude extracts at varying concentration levels using agar plate diffusion to determine the inhibitory effect of *S. commune*. The study took place over the course of S.Y. 2024 - 2025, which started in December 2024 and ended in March 2025. The study only focused on testing on the Gram-positive bacterium *Staphylococcus aureus*. The study did not cover the potential antimicrobial effects on other Gram-positive bacteria, Gram-negative bacteria and other pathogens and the use of different crude extracts aside from aqueous, ethanol and isopropanol. The study also only partially detected all present phytochemicals within *S. commune*.

6. Literature Review

Fungi for General Health Usage. Fungi are highly diverse groups of eukaryotic microorganisms (Morton, 2021). They are present in most habitats, primarily on land (Microbiology Society, n.d.; Barnes et al., 2023). They are essential to the environment's homeostasis, serving as food sources, pathogens, symbionts, saprophytes, and biotic agents (Morton, 2021). Fungi have been cultivated for thousands of years, with the earliest records tracing back to Egyptian and Greek times. In early times, fungi, or mushrooms, were utilized for all kinds of purposes, the most predominant of which is usage for religious purposes (Robinson, 2024; Real Food Encyclopedia - Mushrooms - FoodPrint, 2024). Mushrooms were commonly associated with the divine, possessing properties they believed would bridge the gap between mortal and God, a belief that modern researchers believe is rooted in the hallucinogenic properties of certain mushrooms that activate when consumed (Welle, 2022). Meanwhile, in Asia, countries such as China and Japan have already studied various species of mushrooms for medicinal purposes (Ray et al., 2024). One mushroom of interest, in particular, is the species of *Lentinus edodes*, or the shiitake mushroom. Early records of traditional Chinese medicine refer to mushroom species as an "elixir of life," a substance that can "accelerate vital energy, ward off hunger, cure colds, and defeat body fluid energy" (Studio Animato, n.d.; Rahman & Choudhury, 2013). Other species cultivated

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

early on include the cloud ear mushroom (*Auricularia polytricha*), which has been proven to possess antioxidant, hepatoprotective, antitumor, and immunomodulatory properties (Miao et al., 2020; Islam et al., 2022).

In recent times, fungi have seen an exponential increase in popularity due to many factors, including the general public leaning towards a more healthy lifestyle, the health benefits that the consumption of edible mushrooms offers, the nutritional value provided by mushrooms, and much more (Gibb, 2023; Firebelly Marketing Team, 2024; Ślusarczyk et al., 2021). Moreover, since the first discovery of the antibiotic penicillin from *Penicillium chrysogenum*, fungi have been studied as a potential source of medicinal agents to aid in the healthcare industry (Hutchings et al., 2021; Prescott et al., 2023). Studies have also recognized that mushrooms contain health-promoting properties due to being an excellent source of bioactive compounds, such as terpenoids, steroids, phenols, nucleotides, and their respective derivatives (Assemie et al., 2021; Kumar et al., 2021).

The presence of these bioactive compounds, more specifically, phytochemicals, stimulate biological activities highly sought after by those in the pharmaceutical and healthcare industry, such as antioxidant, antimicrobial, antibacterial, anti-inflammatory, antiallergic, antiviral properties and much more (Gemechu, 2020; Sharma et al., 2020; Ugbogu et al., 2019).

Biological Activities of Split Gill Mushroom (Schizophyllum commune). *S. commune*, or Split Gill mushrooms, is a fungus present nearly everywhere in the world. It commonly grows on the dead branches of deciduous trees (Missouri Department of Conservation, n.d). In the Philippines, it is among the most frequently found wild mushroom species (Dulay et al., 2022), commonly utilised by Indigenous communities for nutraceutical purposes (De Leon et al., 2021). S. commune has been used traditionally as medicine, as communities believed it could serve as a treatment against various diseases and disorders. As per some studies, these beliefs are grounded in reality, as fungi have been found to have a significant amount of phytochemicals, such as alkaloids and flavonoids (Dutta et al., 2023; Chua et al., 2022; Khalil et al., 2020; Shady et al., 2023; Nwanko et al., 2021).

Schizophyllum commune has exhibited antioxidant, anti-inflammatory, and antimicrobial properties (Razak et al., 2024; Prabsangob & Sittiketgorn, 2023; Wongaem et al., 2020; Kumar et al., 2022; Phuket et al., 2019). Another is due to a polysaccharide known as schizophyllan, which is well-known for its antimicrobial, anti-inflammatory, anti-tumour, and antipyretic properties (López-Legarda et al., 2021; Wzorek-Łyczko, 2023; Saetang et al., 2023; Ansari et al., 2024). In line with these findings, methanolic extracts from *S. commune* samples from central Nepal contained a significant amount of phenols, measuring 143.21 ± 0.003 mg gallic acid equivalents (GAE) per 100 grams of extract (Bhatt et al., 2023).

The study by Bhatt et al. aimed to evaluate different phytochemical constituents of two saprophytic mushrooms, namely *Schizophyllum commune* Fr and *Microporus xanthopus* (Fr.) Kuntze, collected from the forest around Phulchowki, Lalitpur, Nepal. The researchers found that *S. commune* had higher total phenolic, flavonoid, vitamin C, β-carotene, and lycopene contents than *M. xanthopus* in methanolic extracts, suggesting potential medicinal value. Other studies have also suggested that *S. commune* can neutralise free radicals, harmful molecules that can damage cells and contribute to disease. (Al-Azad & Ping, 2022). Other studies have also shown that *S. commune* can significantly inhibit the growth of both gram-positive and gram-negative bacteria (Khardziani et al., 2020; Mišković et al., 2021; Acanto & Cuaderes, 2021). *S. commune* has also been seen to be an effective inhibitor of various bacteria due to its ability to resist the creation of biofilms (Hastuty et al., 2020; Sharma et al., 2024). Furthermore, when used to synthesize new products, for example, the usage of gold nanoparticles derived from *S. commune*, it was discovered that *S. commune* possesses great antifungal properties (Alqurashi et al., 2023; Berikashvili et al., 2023). The same antifungal properties were also observed when *S. commune* was grown alongside other fungi (Krause et al., 2020). One such property observed is deadlock, wherein two competing species cannot outgrow the other (Brglez et al., 2020).

Traditional Medicine and Bioactivity of Medicinal Mushrooms. Humans have depended on natural resources for millennia to satisfy essential needs such as food, water, shelter, clothing, and tools. Throughout history, surrounding environments have been explored to enhance quality of life. This exploration involved identifying various organisms with potential benefits, particularly in health. Traditional medicine utilizes natural products such as plants, and modern research continues to investigate different organisms with potential medicinal properties. Mushrooms, in particular, have been a component of traditional medicine for centuries, with studies confirming their bioactivity, showing that mushroom extracts from various species exhibited antidiabetic, antitumor, antihypercholesterolemic, antihypertensive, anti-neurodegenerative, antioxidative, and antimicrobial activities (Luan et al., 2022; Łysakowska et al., 2023; Shevchuk et al., 2023; Stajić et al., 2023).

Cell Wall Resistance of Gram-positive and Gram-negative Bacteria. Bacteria can be either classified as Gram-positive or Gram-negative, based on their differences, particularly the thickness of their cell wall. Their classification

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

can be based on the outcome of a test called the "Gram Stain" (Antibiotic Research UK, 2023). Gram staining involves staining cells with a purple dye (called "crystal violet") that only binds to a substance called peptidoglycan.

The cells are then rinsed and stained with a red dye (safranin); if the cells take on a purple hue, they are deemed "Gram-positive," meaning they contain a high amount of peptidoglycan in their cell wall; if they take on a red hue, they are deemed "Gram-negative" (Tripathi & Sapra, 2023). This is due to the thickness of their cell wall (peptidoglycan); as according to Mai-Prochnow, Gram-positive bacteria are composed of thick cell walls (about 20–80 nm) whereas than Gram-negative bacteria have thin cell walls (<10nm) (Tetteh et al., 2020). This cell wall has a purpose when it comes to resisting foreign materials.

Despite Gram-positive bacteria having thicker cell walls than Gram-negative bacteria, antibiotics can readily reach the peptidoglycan in Gram-positive bacteria. This is not possible for Gram-negative bacteria, as they have an outer membrane that acts as a protective layer and is necessary for their survival (Tetteh et al., 2020). If any alteration occurs the outer membrane of the gram-negative bacteria, creates a stronger resistance as it releases endotoxins (lipopolysaccharides), leading to severe immune responses and potential sepsis, along with their inherent and acquired antibiotic resistance (Breijyeh et al., 2020).

In addition to that, gram-positive bacteria don't have an outer membrane, which makes them more vulnerable to staining and antibiotics, and because their thick peptidoglycan cell wall is more absorbent, which helps with infection detection and treatment, gram-positive bacteria are typically simpler to study than gram-negative bacteria (Breijyeh et al., 2020). One example of Gram-positive bacteria is *Staphylococcus aureus* (most popular Gram-positive pathogen), it is a bacterium that belongs to the group collectively known as ESCAPE pathogens, which is considered as the most serious bacterial infections occur in health care settings (Tetteh et al., 2020). These pathogens' infections are mostly challenging to treat because of rising resistance and a lack of effective treatment options (Tetteh et al., 2020).

Bioactive Compound Extraction using Ethanol, Isopropyl and Aqueous Crude Extracts. A study by Harish et al. (2024) analyzes the bioactive compounds present in the Neem tree (*Azadirachta indica*) using isopropyl alcohol and ethanol extracts, which identified alkaloids, flavonoids, terpenoids, and quinones in both solvents, with isopropyl alcohol additionally containing phenols and glycosides. These findings highlight the effectiveness of ethanol and isopropyl alcohol in extracting bioactive compounds with potential antibacterial and anti-inflammatory properties.

Ethanol's versatility in extracting both polar and moderately non-polar compounds makes it a preferred solvent for phytochemical extraction. Isopropyl alcohol's higher polarity and boiling point enhance extraction yields, particularly for cannabinoids, though it may also extract higher levels of chlorophyll (Plaskova & Mlcek, 2023).

In addition, aqueous solutions are also highly effective for extracting polar bioactive compounds. A study by Mondal (2025) demonstrated their efficiency in extracting heavy metals like lead, strontium, zinc, and cadmium using *S. commune*, with maximum biosorption capacities of 81.5, 90.71, 70.18, and 66.18 mg/g.

Concentration Levels of Fungi Crude Extracts for Antimicrobial Assays. The study of Acanto & Cuaderes (2021) aimed to investigate the antimicrobial activity and phytochemical composition of ethanolic extracts from the Split Gill mushroom (*Schizophyllum commune*). To be able to determine the inhibition rate of *S. commune* against the selected Gram-positive bacteria and Gram-negative bacteria, the researchers used an ethanolic extract with different concentrations (100%, 75%, 50%, and 25%) through diluting the concentrated extract with sterile distilled water for antibacterial assays.

Another study was conducted also by Al-Azad & Ping (2022), which compares the antioxidant and antimicrobial properties of extracts from two edible mushrooms: *Pleurotus sajor-caju* (commercial) and *Schizophyllum commune* (wild). The researchers used aqueous extracts and three organic solvent extracts (50% ethanol, methanol, and acetone) to assess these properties. There is a lack of studies utilizing other extract concentration levels, such as 80% concentration for crude extracts, making it a novel approach to explore its potential efficacy.

7. Methods

Study Design. This study utilized a quantitative research design, following an experimental approach. The experimental quantitative research approach best fitted this study because the study sought to conduct a screening of the phytochemical constituents and the antimicrobial properties of Split Gill mushroom crude extracts when tested against *Staphylococcus aureus*.

Materials. For this study, the primary material that was utilized to gain results was the Split Gill mushroom (*S. commune*). The researchers collected and stored samples of the Split Gill mushroom (*S. commune*) from various parts of Negros Occidental. Majority of the samples were extracted from Don Salvador Benedicto, Negros Occidental, with the rest being collected from Bacolod City and Bago City. Zip-lock bags were used to store the collected samples of

BTTHS Journal of
Students' Projects in Advancing Research
Knowledge (S.P.A.R.K)

the mushroom and labeled accordingly. The samples of the Split Gill mushroom were then stored in a chilled environment before its preparation for the phytochemical screening and antimicrobial assay. The samples of the mushroom were transferred to Negros Prawn Producers Cooperative to be dried using a drying oven for 7 days.

Once the drying process was complete, the extraction process began, starting with maceration and then with rotary evaporation. A blender, Erlenmeyer flasks, Whatman No.1 filter paper, and glass stirring rods were used for the maceration phase of the extraction. In the rotary evaporation phase, round bottom flasks, a cold trap, and a bump trap were utilized.

Once the solute had undergone rotary evaporation, phytochemical screening was conducted via a series of tests to check for any possible phytochemical constituents within *S. commune*. After the screening, an antimicrobial assay was performed on *Staphylococcus aureus*.

Equipments. The equipment utilized in maceration is a drying oven. A rotary evaporator and a heated water bath were used in the rotary evaporation phase. For the antimicrobial assay, a ventilated duct fume hood and a freezer were used.

Procedures. Before the screening and intervention were administered to the experimental groups, the researchers began by obtaining and authenticating Split Gill mushroom (*Schizophyllum commune*) samples via macroscopic analysis through existing literature regarding mushrooms. Once the collected samples matched that of the descriptions of *S. commune* within literature, clear photographs of the mushrooms were taken and uploaded to the iNaturalist database, which is an online social network for information on biodiversity through the sharing of plant and animal species. The fungi's classification and scientific name were confirmed by Eugene Popov, a verified mycologist and researcher, who deemed the samples as Research Grade. After that, extraction proceeded.

During the extraction process, the collected samples of *Schizophyllum commune* were mixed with aqueous, ethanol, and isopropyl solutions to create crude extracts. Phytochemical screening was conducted to identify the possible phytochemical constituents within *S. commune*. Subsequently, an antimicrobial assay was performed on the selected gram-positive bacterium *S. aureus* to determine the inhibitory effects, if any, of the different extracts of Split Gill mushroom. The antimicrobial assay that was conducted is the Kirby-Bauer or Disc Diffusion method.

Before the tests were performed, McFarland's Standard was prepared. 100 mL of McFarland's standard was prepared by mixing 99.5 mL of 1% sulfuric acid and 0.5 mL of 1.175% BaCl2 • 2H2O. The turbidity is equivalent to 1.0 x 108 CFU/ml bacteria. Bacterial isolates from pure culture of *S. aureus* was then transferred to sterile buffered phosphate or distilled water solution and the turbidity was adjusted using a spectrophotometer at 600 nm wavelength in comparison with the absorbance of the McFarland's standard, 0.089.

To conduct the Kirby-Bauer test, first, a sterile swab was dipped into the broth culture of *S. aureus* and was gently squeezed against the inside of the tube in order to remove excess fluid in the swab. The swab with the S. aureus was streaked evenly on each of the sterile and dried Mueller-Hintor agar (MHA) plates. After the streaking was completed, the plates were made to dry for 5 minutes. Then, paper discs were prepared and impregnated with the distilled water, ethanol, isopropanol crude extracts of *S. commune*, Gentamicin that served as the positive control, and sterile distilled water that served as the negative control. After the drying, the discs from the positive (+) controls (Gentamicin) and negative (-) control (sterile distilled water), and the different extracts of *S. commune* were placed on the surface of each of the agar using sterilized forceps. The discs were gently pressed into the surface of the agar using flame sterilized forceps. The plates were then carefully incubated for 24 hours at 37°C. After incubation, a caliper was used to measure the diameter of the zone of inhibition for each of the discs used.

The measurements obtained from the individual discs were recorded and compared with the standard table to determine the sensitive zone and whether the tested bacterial species are sensitive to the different extracts of *S. commune* and the controls used.

After the experiments were executed, the collected data were encoded and analyzed, which will be described in the next section. The researchers used JASP version 0.19.1 to analyze the data gathered.

For Problem Statement 1, descriptive analysis was utilized to determine the phytochemicals present in *S. commune*. For Problem Statement 2, mean and standard deviation were used to determine the zone of inhibition of *S. commune* against *Staphylococcus aureus*. For Problem Statement 3, Kruskal-Wallis and Dunn's Test were used to determine the significant difference between the crude extracts' zones of inhibition.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

8. Results and Discussion

Table 1
Phytochemical Compounds in Split Gill Mushroom (Schizophyllum commune)

Test	Aqueous Extract	Positive Reaction
Alkaloids	+	Creamy white precipitate
Tannins	+	Dark green or black precipitate
Saponin	+	Continued frothing
Proteins	+	Violet or pink coloration
Phenols	+	Bluish green or black coloration
Flavonoids	+	Yellow coloration
Glycosides	+	Upper layer - bluish green color Lower layer - brownish red color
Carbohydrates	+	Brick red precipitate
Terpenoids	+	Reddish brown interface

Note: (+) - Present, (-) - Not Present

The phytochemical compound analysis of *S. commune* using aqueous extract revealed the presence of all phytochemical parameters: alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids. This is in partial agreement with the study of Acanto & Cuaderes (2021) and Bhatt et al. (2023). However, the former tested negative for glycosides, contradicting the present findings.

Table 2
Inhibition Rates of Split Gill Mushroom (S. commune) Crude Extracts against Staphylococcus aureus in Various Crude Extracts

Concentration (80%)	N	Mean (mm)	SD	Interpretation
S. commune Aqueous Extract	10	4.800	4.131	Resistant
S. commune Ethanolic Extract	10	10.200	0.789	Resistant
S. commune Isopropyl Extract	10	8.600	0.516	Resistant

Each of the crude extracts of *S. commune* exhibited minimal inhibitory activity, with a mean zone of inhibition of 4.8 mm, 10.2 mm, and 8.6 mm for the aqueous, ethanolic, and isopropyl extracts respectively. According to the protocols provided by Hudzicki (2009) from the American Society of Microbiology, bacteria belonging to the *Staphylococcus* species were considered to show resistance towards an antibiotic (Gentamicin) when the zone of inhibition is measured to be less than 12 mm in diameter. Based on these standards, the crude extracts of *S. commune* were not effective inhibitors of the Gram-positive bacterium *S. aureus*.

Regarding inhibition zones, the ethanol crude extract of *S. commune* had the largest zone of inhibition, implying relatively stronger antimicrobial activity than its counterparts. It was followed by the isopropanol crude extract, then the aqueous extract. This was consistent with the findings of Acanto & Cuaderes (2021) and Al-Azad & Ping (2022) which showed that ethanol extracts displayed the highest activity and inhibition zones in comparison to other extracts like methanol or acetone.

However, the average inhibition zones listed in the table above are lower than that of Acanto & Cuaderes (2021), whose ethanolic extract of *S. commune* had a mean zone of inhibition measuring 26.14 mm at 75% concentration when tested against *Staphylococcus aureus*. Khardziani et al. (2020) also concluded that *S. commune* had strong inhibitory activity, with their results displaying a 17±1 mm zone of inhibition diameter against *S. aureus*. The researchers inferred that the disparity between the results of this study and that of related literature was due to the amount of mushroom samples used for the experimentation.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Table 3
Difference Between the Inhibition Rates of Split Gill Mushroom (S. commune) Crude Extracts against Staphylococcus aureus in Various Crude Extracts

Factor	Statistic	df	p
Extracts	22.859	2	<0.001

The obtained p-value <0.001 was less than the $\alpha = 0.05$. Therefore, there was a significant difference between the inhibition rate of *S. commune* against *Staphylococcus aureus* in the following concentrations: 80% concentration of aqueous crude extract of *S. commune*, 80% concentration of ethanol crude extract of *S. commune*, 80% concentration of isopropanol crude extract of *S. commune*. Thus, the researchers rejected the null hypothesis.

Table 4
Comparison of the Difference Between the Inhibition Rates of Split Gill Mushroom (S. commune) Crude Extracts against Staphylococcus aureus in Various Crude Extracts

Comparison	z	p
S. commune Ethanolic Extract (80%) – *S. commune* Isopropyl Extract (80%)	2.623	0.009
S. commune Ethanolic Extract (80%) – *S. commune* Aqueous Extract (80%)	4.773	<0.001
S. commune Isopropyl Extract (80%) – *S. commune* Aqueous Extract (80%)	2.151	0.032

The p-value of ethanolic extract versus isopropyl extract was 0.009 with a z-score of 2.623. In statistics, if the p-value is less than 0.05, it is typically considered significant, while a p-value greater than 0.05 is considered not significant. Since p <0.05 in this case, it indicated that these two extracts had statistically different effects.

Ethanolic extract versus aqueous extract had a p-value less than 0.001 and a z-score of 4.773. There was a large difference when comparing the p-values of 0.001 and 0.05, suggesting that the ethanolic extract was highly significantly different from the aqueous extract.

Isopropyl extract versus aqueous extract had a p-value of 0.032 with a z-score of 2.151. Since p <0.05, there was a significant difference between the isopropyl and aqueous extracts of *S. commune*.

9. Conclusions and Recommendations

Conclusions

The results of this study contributed to a growing body of evidence supporting the potential of *S. commune* as an antimicrobial agent.

The findings of the Phytochemical Assay of Split Gill mushroom (*S. commune*) aqueous extract confirmed the presence of various bioactive compounds. Furthermore, the results of the Kirby-Bauer Disk Diffusion Test of Split Gill mushroom (*S. commune*) aqueous, ethanol, and isopropyl extracts against *S. aureus* showed that, of the three crude extracts, the extract with the highest inhibition rate was the ethanolic crude extract. Therefore, it could be concluded that to gain the highest inhibition rate against *Staphylococcus aureus* using *S. commune*, it should be extracted using an ethanolic solution. However, given that the inhibition rate shown in the results was lower than that of existing literature, it can be inferred that a higher amount of mushrooms must be added into the extract to make its effects more potent.

Finally, after evaluating the difference in inhibition rates of Split Gill mushroom (*S. commune*) against *Staphylococcus aureus* in various crude extracts, the results showed a strong significant difference in zone of inhibition diameters across the different crude extracts of *S. commune*. This suggests that *S. commune's* antimicrobial activity varies significantly depending on the extraction solvent used.

Recommendations

Considering the final data, interpretation, and conclusions, the recommendations are briefly discussed. Environmentalists and indigenous communities can advocate for the conservation and use of traditional methods as an affordable and eco-friendly alternative to medicine. Healthcare professionals, the Department of Environment and Natural Resources, and the Department of Health can incorporate the findings of this study into alternative treatments.

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Future researchers can expand the existing literature by exploring the antimicrobial properties of *S. commune* further using other solvents, concentration levels, and different bacteria.

10. Declaration of Conflict of Interest

Authors have declared that no competing interests exist.

11. Literatures Cited

Acanto, R., & Cuaderes, V. H. (2021). Antimicrobial Activity And Phytochemical Screening Of Split Gill Mushroom (Schizophyllum Commune) Ethanolic Extract. *International Journal of Scientific & Technology Research*, 10(10). https://www.ijstr.org/final-print/oct2021/Antimicrobial-Activity-And-Phytoche mical-Screening-Of-Split-Gill-Mushroom-schizophyllum-Commune-Ethanol ic-Extract.pdf

Acanto, R. B., Cuaderes, V. H. S., & Gimoto, P. H. (2022). *Phytochemical Screening, Cytotoxic Activity, and Proximate Analysis of Split Gill Mushroom (Schizophyllum commune)*. https://ejournals.ph/article.php?id=18067&webview_progress_bar=1&sho w_loading=0

Agarwal, A., Gupta, V., Yadav, A. N., Sain, D., Rahi, R. K., Bera, S. P., & Neelam, D. (2023). Aspects of mushrooms and their extracts as natural antimicrobial agents: Microbiology. *Journal of Microbiology, Biotechnology and Food Sciences*, 12(6), e9191. https://doi.org/10.55251/jmbfs.9191

Al-Azad, S., & Ping, V. C. A. (2022). Antioxidant Properties and Antimicrobial Activity in the Extracts of Two Edible Mushroom, Pleurotus sajor caju and Schizophyllum commune. *Advances in Bioscience and Biotechnology*, 13(09), 352–361. https://doi.org/10.4236/abb.2022.139023

Al-Garadi, M., Qaid, M., Alqhtani, A., Alhajj, Al-Abdullatif, A., & Al-Mufarrej, S. (2023). In Vitro Antimicrobial Efficacy Assessment of Ethanolic and Aqueous Extracts of Cinnamon (Cinnamomum Verum) Bark against Selected Microbes. *Brazilian Journal of Poultry Science*, 25(1). https://doi.org/10.1590/1806-9061-2022-1682

Alqurashi, Y. E., Almalki, S. G., Ibrahim, I. M., Mohammed, A. O., Abd El Hady, A. E., Kamal, M., Fatima, F., & Iqbal, D. (2023). Biological synthesis, characterization, and therapeutic potential of S. commune-mediated gold nanoparticles. *Biomolecules*, 13(12), 1785. https://doi.org/10.3390/biom13121785

Ansari, E., Alvandi, H., Kianirad, S., Hatamian-Zarmi, A., & Mokhtari-Hosseini, Z. B. (2024). Research progress on production and biomedical applications of Schizophyllan as a tailor-made polysaccharide: A review. *Carbohydrate Polymers*, 122770. https://doi.org/10.1016/j.carbpol.2024.122770

Aryal, S. (2022, August 10). Potato dextrose Agar (PDA)- *Principle, uses, composition, procedure and colony characteristics*. Microbiology Info.com. https://microbiologyinfo.com/potato-dextrose-agar-pda-principle-uses-com position-procedure-and-colony-characteristics/

Assemie, A., & Abaya, G. (2022). The effect of edible mushroom on health and their biochemistry. *International Journal of Microbiology*, 2022, 1–7. https://doi.org/10.1155/2022/8744788

Baracco, G. J. (2019). Infections caused by Group C and G Streptococcus (Streptococcus dysgalactiae subsp. equisimilis and others): Epidemiological and clinical aspects. *Microbiology Spectrum*, 7(2). https://doi.org/10.1128/microbiolspec.gpp3-0016-2018

Barnes, A., Barrera, A. C., Chamberlain, J., Foranoce, L., & Fulfaro, J. (Eds.) (2023). Schizophyllum commune. *Microbe Wiki*. https://microbewiki.kenyon.edu/index.php/Schizophyllum_commune

Berikashvili, V., Khardziani, T., Kobakhidze, A., Kulp, M., Kuhtinskaja, M., Lukk, T., Gargano, M. L., Venturella, G., Kachlishvili, E., Metreveli, E., Elisashvili, V. I., & Asatiani, M. (2023). Antifungal Activity of Medicinal Mushrooms and Optimization of Submerged Culture Conditions for Schizophyllum commune (Agaricomycetes). *International Journal of Medicinal Mushrooms*, 25(10), https://doi.org/10.1615/intjmedmushrooms.2023049836

Bhandari, P. (2023, June 22). *What Is Quantitative Research? | Definition, Uses & Methods*. Scribbr. Retrieved October 15, 2024, from https://www.scribbr.com/methodology/quantitative-research/

Bhat, A. (2023, November 24). *Experimental Research: What it is + Types of designs*. QuestionPro. https://www.questionpro.com/blog/experimental-research/

Bhatia, A., Bhatia, J.N., & Neeraj, B. (2024). Medicinal mushrooms and their impact on human health. *Food and Scientific Reports*, 5(1), 38-43. https://www.researchgate.net/publication/377985905_Medicinal_Mushroo ms_and_their_Impact_on_Human_Health

Bhatia, J. (2024, June 21). Gram-positive bacteria: Introduction, characteristics, benefits. *PHYSICS* https://www.pw.live/exams/school/gram-positive-bacteria/

Bhatt, L. R., Dawadi, P., Shyaula, M., Khadka, C., & Raut, J. K. (2023). Phytochemical analysis of Schizophyllum

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

commune Fr. and Microporus xanthopus (Fr.) Kuntze from Phulchowki, central Nepal. *Scientific World*, 16(16), 100–105. https://doi.org/10.3126/sw.v16i16.56867

Bhattacharya, A. (2019). High-Temperature stress and metabolism of secondary metabolites in plants. In *Elsevier eBooks* (pp. 391–484). https://doi.org/10.1016/b978-0-12-817562-0.00005-7

Bobbitt, Z. (2020, September 30). *Dunn's Test for Multiple Comparisons*. Statology. https://www.statology.org/dunns-test/

Booq, R. Y., Alshehri, A. A., Almughem, F. A., Zaidan, N. M., Aburayan, W. S., Bakr, A. A., Kabli, S. H., Alshaya, H. A., Alsuabeyl, M. S., Alyamani, E. J., & Tawfik, E. A. (2021). Formulation and Evaluation of Alcohol-Free Hand Sanitizer Gels to Prevent the Spread of Infections during Pandemics. *International Journal of Environmental Research and Public Health*, 18(12), 6252. https://doi.org/10.3390/ijerph18126252

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-Negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, 25(6), 1340. https://doi.org/10.3390/molecules25061340

Brglez, A., Piškur, B., & Ogris, N. (2020). In vitro interactions between Eutypella parasitica and some frequently isolated fungi from the wood of the dead branches of young sycamore maple (Acer pseudoplatanus). *Forests*, 11(10), 1072. https://doi.org/10.3390/f11101072

Bush, L. M. (2023, March 10). Overview of Gram-Positive bacteria. *MSD Manual Consumer Version*. https://www.msdmanuals.com/home/infections/bacterial-infections-gram-p ositive-bacteria/overview-of-gram-positive-bacteria

Cambridge University Press. (2024). https://dictionary.cambridge.org/dictionary/english/steamer

Chemical Entities of Biological Interest. (n.d.-a). *Ethanol*. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16236

Chemical Entities of Biological Interest. (n.d.-b). *Propan-2-OL*. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:17824

Chua, R. W., Song, K. P., & Ting, A. S. Y. (2022). Antimicrobial activities and phytochemical screening of endophytic fungi isolated from Cymbidium and Dendrobium orchids. *South African Journal of Botany*, 151, 909–918. https://doi.org/10.1016/j.sajb.2022.11.015

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, 284(5418), 1318–1322. https://doi.org/10.1126/science.284.5418.1318

Das, A. K., Islam, M. N., Faruk, M. O., Ashaduzzaman, M., & Dungani, R. (2020). Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*, 135, 58–70. https://doi.org/10.1016/j.sajb.2020.08.008

Dasanayaka, P., & Wijeyaratne, S. (2017). Cultivation of Schizophyllum commune mushroom on different wood substrates. *Journal of Tropical Forestry and Environment*, 7(1). https://doi.org/10.31357/jtfe.v7i1.3023

De La Fuente-Núñez, C., Reffuveille, F., Fernández, L., & Hancock, R. E. (2013). Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology*, 16(5), 580–589. https://doi.org/10.1016/j.mib.2013.06.013

De Leon, A., Pagaduan, M. A., Panto, B., & Kalaw, S. (2021). Species listing of macrofungi found in Paracelis Mountain Province, Philippines. *CLSU International Journal of Science and Technology*, 5(2), 22–40. https://doi.org/10.22137/ijst.2021.v5n2.03

Deka, J., Mahanta, J., Ahmed, H., Tamuly, S., & Choudhury, D. (2020). Effect of dietary supplementation of neem leaf powder (Azadirachta indica) on the haematological and biochemical parameters of commercial broiler chicken. *International Journal of Chemical Studies*, 8(4), 78–81. https://doi.org/10.22271/chemi.2020.v8.i4b.9866

Du, B., Yang, Y., Bian, Z., & Xu, B. (2017). Characterization and Anti-Inflammatory Potential of an Exopolysaccharide from Submerged Mycelial Culture of Schizophyllum commune. *Frontiers in Pharmacology*, 8. https://doi.org/10.3389/fphar.2017.00252

Dulay, R. M. R., Batangan, J. N., Kalaw, S. P., De Leon, A. M., Cabrera, E. C., Kimura, K., Eguchi, F., & Reyes, R. G. (2022). Records of wild mushrooms in the Philippines: A review. *Journal of Applied Biology & Biotechnology*. https://doi.org/10.7324/jabb.2023.110202

Dutta, R., Ghosal, S., Roy, S., Bhattacharyya, D. K., & Bhowal, J. (2023). Phytochemical screening and antioxidative property evaluation of lipid-producing fungi. *Archives of Microbiology*, 205(2). https://doi.org/10.1007/s00203-022-03399-3

Duttaroy, A. K. (2021). Health effects of terpenoids. In *Elsevier eBooks* (pp. 413–424). https://doi.org/10.1016/b978-

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

0-12-822405-2.00017-7
Elgie, K. (2022). What is a rotary evaporator? Asynt. https://www.asynt.com/blog/what-is-a-rotary-evaporator/
Firebelly Marketing Team. (2024, February 28). *Why Mushrooms are Trending In The Food and Beverage Industry: Consumers Crave Adaptogenic Magic – Firebelly Marketing*. Firebelly Marketing – Social Media Marketing Agency. https://www.firebellymarketing.com/blog/why-mushrooms-are-trending-in-t he-food-and-beverage-industry-consumers-crave-adaptogenic-magic
Flavonoids. (2025). Linus Pauling Institute. https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/flavonoids
Froilabo. (2022). Heated laboratory water bath function and uses. https://www.froilabo.com/blog/heated-laboratory-water-bath-function-and-uses/
Froilabo. (2024, March 21). What is a laboratory incubator? https://www.froilabo.com/blog/what-is-a-laboratory-incubator/
Gemechu, F. G. (2020). Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. *Trends in Food Science & Technology*, 104, 235–261. https://doi.org/10.1016/j.tifs.2020.08.005
Gibb, A. L. (2023, December 5). *Mushrooms take center stage in 2024 food trends*. https://www.linkedin.com/pulse/mushrooms-take-center-stage-2024-food-t rends-alexandra-leung-jgw3c/
Harish, A., Sekar, R. R. J., & Vijila, S. (2024). GC-MS Analysis of Bioactive Compounds in Azadirachta indica Extracts and their Antibacterial Effect against Fish Pathogen Aeromonas hydrophila. *Uttar Pradesh Journal of Zoology*, https://doi.org/10.56557/upjoz/2024/v45i144196
Haslam, D. B., & St Geme, J. W. (2022). Groups C and G streptococci. *Principles and Practice of Pediatric Infectious Diseases* (6th ed.), 752-753. https://doi.org/10.1016/b978-0-323-75608-2.00122-1
Hastuty, A., Mairani, R., & Rosada, K. K. (2020). Antibacterial and Anti-Biofilm Activities of Culture Filtrates from Schizophyllum commune, Coniothyrium sp., and Fusarium sp. https://www.semanticscholar.org/paper/Antibacterial-and-Anti-Biofilm-Activ ities-of-from-Hastuty-Mairani/c0078ac170782861ac7465ff8e793a3279892 5b3
Haurowitz, F., & Koshland, D. (2025, February 14). Protein | Definition, Structure, & Classification. Encyclopedia https://www.britannica.com/science/protein
History of mushroom cultivation - Lowimpact.org. (2015, June 2). Lowimpact.org. https://www.lowimpact.org/categories/mushroom-cultivation/further-info/his tory-of-mushroom-cultivation
Hudzicki, J. (2009) Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/ Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf
Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, 51, 72–80. https://doi.org/10.1016/j.mib.2019.10.008
Islam, T., Yao, F., Kang, W., Lu, L., & Xu, B. (2022). A systematic study on mycochemical profiles, antioxidant, and anti-inflammatory activities of 30 varieties of Jew's ear (Auricularia auricula-judae). *Food Science and Human Wellness*, https://doi.org/10.1016/j.fshw.2022.03.005
Jain, N. (2023, July 7). What is quantitative research design? Definition, types, methods, and best practices. *IdeaScale*. https://ideascale.com/what-is-quantitative-research-design/
Katoch, R., Tripathi, A., & Kiran, A. (2023). Rice bean (Vigna umbellata). In *Elsevier eBooks* (pp. https://doi.org/10.1016/b978-0-323-90537-4.00002-8
Khalil, D., El-Zayat, S. A., & El-Sayed, M. A. (2020). Phytochemical Screening and Antioxidant Potential of Endophytic Fungi Isolated From Hibiscus sabdariffa. *Journal of Applied Biotechnology Reports*, 7(2), 116–124. https://doi.org/10.30491/jabr.2020.109287
Khan, S., Sahar, A., Tariq, T., Sameen, A., & Tariq, F. (2023). Essential oils in plants: Plant physiology, the chemical composition of the oil, and natural variation of the oils (chemotaxonomy and environmental effects, etc.). In *Elsevier eBooks* (pp. https://doi.org/10.1016/b978-0-323-91740-7.00016-5
Khardziani, T., Metreveli, E., Didebulidze, K., & Elisashvili, V. I. (2020). Screening of Georgian Medicinal Mushrooms for Their Antibacterial Activity and Optimization of Cultivation Conditions for the Split Gill Medicinal Mushroom, Schizophyllum commune BCC64 (Agaricomycetes). *International Journal of Medicinal Mushrooms*, 22(7), 659–669. https://doi.org/10.1615/intjmedmushrooms.2020035051
Kotkar, G. D., Shetgaonkar, A. D., & Tilve, S. G. (2023). Diversity of chemical skeletons. In *Elsevier eBooks* https://doi.org/10.1016/b978-0-443-15232-0.00023-0

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Krause, K., Jung, E., Lindner, J., Hardiman, I., Poetschner, J., Madhavan, S., Matthäus, C., Kai, M., Menezes, R. C., Popp, J., Svatoš, A., & Kothe, E. (2020). Response of the wood-decay fungus Schizophyllum commune to co-occurring microorganisms. *PLoS ONE*, 15(4), e0232145. https://doi.org/10.1371/journal.pone.0232145

Kumar, A., Kumar, M., Ali, S., Lal, S.B., & Sinha, M.P. (2019). Anti-pathogenic efficacy of Indian edible macrofungi Dacryopinax spathularia (Schwein) and Schizophyllum commune (Fries) against some human pathogenic bacteriae. *Journal for Emerging Technologies and Innovative Research*, 6(1), 695-703. https://www.researchgate.net/publication/336232485_Antipathogenic_efficacy_of_Indian_edible_macrofungi_Dacryopinax_spathularia_Schwein_a nd_Schizophyllum_commune_Fries_against_some_human_pathogenic_bacteriae

Kumar, K., Mehra, R., Guiné, R. P. F., Lima, M. J., Kumar, N., Kaushik, R., Ahmed, N., Yadav, A. N., & Kumar, H. (2021). Edible Mushrooms: A Comprehensive Review on Bioactive Compounds with Health Benefits and Processing Aspects. *Foods*, 10(12), 2996. https://doi.org/10.3390/foods10122996

Kumar, A., Bharti, A. K., & Bezie, Y. (2022). Schizophyllum commune: A fungal cell-factory for production of valuable metabolites and enzymes. *BioResources*, 17(3), 5420–5436. https://doi.org/10.15376/biores.17.3.kumar

Kurnia, F., Nadi, P., & Miati, N. (2020). Isolation of Schizophyllum commune from Rubberwood. *International Journal of Current Microbiology and Applied Sciences*, 9(12), 1202–1207. https://doi.org/10.20546/ijcmas.2020.912.147

LAB 797. (2021, July 21). How do we incubate samples? https://www.lab797.com/blog/2021/5/25/incubation-101

Laboratory Cognizance. (2023, June 7). Potato dextrose agar: Fungal Culture Media. laboratorycognizance.com. https://laboratorycognizance.com/2023/04/potato-dextrose-agar-the-cultur e-media-for-fungal-isolation-and-enumeration/#What_is_Potato_Dextrose _Agar

Libretexts. (2021). 1.10: *Carbohydrates*. Biology LibreTexts. https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/I ntroductory_Biology_(CK-12)/01%3A_Introduction_to_Biology/1.10%3A_ Carbohydrates

López-Legarda, X., Rostro-Alanis, M., Parra-Saldivar, R., Villa-Pulgarín, J. A., & Segura-Sánchez, F. (2021). Submerged cultivation, characterization and in vitro antitumor activity of polysaccharides from Schizophyllum radiatum. *International Journal of Biological Macromolecules*, 186, 919-932. https://doi.org/10.1016/j.ijbiomac.2021.07.084

Luan, F., Peng, X., Zhao, G., Zeng, J., Zou, J., Rao, Z., Liu, Y., Zhang, X., Ma, H., & Zeng, N. (2022). Structural diversity and bioactivity of polysaccharides from medicinal mushroom Phellinus spp.: A review. *Food Chemistry*, 397, 133731. https://doi.org/10.1016/j.foodchem.2022.133731

Łysakowska, P., Sobota, A., & Wirkijowska, A. (2023). Medicinal Mushrooms: Their bioactive Components, Nutritional Value and Application in Functional Food Production—A Review. *Molecules*, 28(14), 5393. https://doi.org/10.3390/molecules28145393

Masyita, A., Sari, R. M., Astuti, A. D., Yasir, B., Rumata, N. R., Emran, T. B., Nainu, F., & Simal-Gandara, J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry X*, 13, 100217. https://doi.org/10.1016/j.fochx.2022.100217

Mello, T. P., Bittencourt, V. C. B., Liporagi-Lopes, L. C., Aor, A. C., Branquinha, M. H., & Santos, A. L. (2018). Insights into the social life and obscure side of Scedosporium/Lomentospora species: ubiquitous, emerging and multidrug-resistant opportunistic pathogens. *Fungal Biology Reviews*, 33(1), 16–46. https://doi.org/10.1016/j.fbr.2018.07.002

Merriam-Webster. (n.d.). Distilled water. In Merriam-Webster.com dictionary. Retrieved October 24, 2024, from https://www.merriam-webster.com/dictionary/distilled%20water

Miao, J., Regenstein, J. M., Qiu, J., Zhang, J., Zhang, X., Li, H., Zhang, H., & Wang, Z. (2020). Isolation, structural characterization and bioactivities of polysaccharides and its derivatives from Auricularia-A review. *International Journal of Biological Macromolecules*, 150, 102–113. https://doi.org/10.1016/j.ijbiomac.2020.02.054

Microbiology Society. (n.d.). Fungi | What is microbiology? https://microbiologysociety.org/why-microbiology-matters/what-is-microbiol ogy/fungi.html

Midpeninsula Regional Open Space District. (n.d.). Split gill mushroom. https://www.openspace.org/where-to-go/nature/split-gill-mushroom

Mišković, J., Karaman, M., Rašeta, M., Krsmanović, N., Berežni, S., Jakovljević, D., Piattoni, F., Zambonelli, A.,

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Gargano, M. L., & Venturella, G. (2021). Comparison of Two Schizophyllum commune Strains in Production of Acetylcholinesterase Inhibitors and Antioxidants from Submerged Cultivation. *Journal of Fungi*, 7(2), 115. https://doi.org/10.3390/jof7020115

Missouri Department of Conservation. (n.d.). Common Split Gill. https://mdc.mo.gov/discover-nature/field-guide/common-split-gill

Mondal, P. (2025). Exploring Schizophyllum commune for sustainable mycoremediation of heavy metals. *Journal of Advanced Agricultural Research*, 1(1), 18–21.

Morton, J. B. (2021). Fungi. In *Elsevier eBooks* (pp. 149–170). https://doi.org/10.1016/b978-0-12-820202-9.00006-x

MRC Laboratory Equipment. (n.d.). What is rotary evaporator? https://www.mrclab.com/what-is-rotary-evaporator

National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 996, Phenol. https://pubchem.ncbi.nlm.nih.gov/compound/Phenol.

Naz, K., Ullah, N., Zaheer, T., Shehroz, M., Naz, A., & Ali, A. (2020). Pan-genomics of model bacteria and their outcomes. *Pan-genomics: Applications, Challenges, and Future Prospects*, https://doi.org/10.1016/b978-0-12-817076-2.00009-3

NCI Dictionary of Cancer Terms. (n.d.). Definition of antimicrobial. Cancer.gov. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/antimicrobial

NL Scientific. (n.d.). Laboratory drying oven. https://nl-test.com/news/laboratory-drying-oven

Nori, P., Nadeem, N., Saraiya, N., Szymczak, W., Boland-Reardon, C., & Kahn, M. (2020). Beta-hemolytic group a streptococcal orthopaedic infections: Our institutional experience and review of the literature. *Journal of Orthopaedics*, 21, 150–154. https://doi.org/10.1016/j.jor.2020.03.015

Nwankwo, O. L., Chukwuebuka, O. C., Collins, O. O., Samuel, B. J., Obasi, J. C., Iloh, E. S., & Nwankwo, E. O. (2021). Quantitative phytochemical analysis of the fungus endophytic extracts isolated from Azadirachta indica using gas chromatography- flame ionization detector. *Journal of Drug Delivery and Therapeutics*, 11(5), 80–83. https://doi.org/10.22270/jddt.v11i5.4999

Pandita, D., Das, A., Jain, G. K., Agarwal, P., Grewal, A. S., Khar, R. K., & Lather, V. (2021). Role of phytoconstituents in the management of COVID-19. *Chemico-Biological Interactions*, 341, 109449. https://doi.org/10.1016/j.cbi.2021.109449

Phuket, S. R. N., Sangkaew, T., Chanapan, P., & Techaoei, S. (2019). Biological activity of SS-Glucans from edible mushroom Schizophyllum commune in Thailand. *International Journal of Applied Pharmaceutics*, 110–112. https://doi.org/10.22159/ijap.2019.v11s5.t0083

Plaskova, A., & Mlcek, J. (2023). New insights of the application of water or ethanol-water plant extract rich in active compounds in food. *Frontiers in Nutrition*, 10. https://doi.org/10.3389/fnut.2023.1118761

Prabsangob, N., & Sittiketgorn, S. (2023). Profile of taste-related compounds and bioactivity of split gill mushroom (Schizophyllum commune) as affected by blanching and drying. *International Journal of Food Properties*, 26(1), 2078–2090. https://doi.org/10.1080/10942912.2023.2243054

Prescott, T. A. K., Hill, R., Mas-Claret, E., Gaya, E., & Burns, E. (2023). Fungal drug discovery for Chronic disease: History, new discoveries and New approaches. *Biomolecules*, 13(6), 986. https://doi.org/10.3390/biom13060986

Rahman, T., & Choudhury, M. (2013). Shiitake mushroom: a tool of medicine. *Bangladesh Journal of Medical Biochemistry*, 5(1), 24–32. https://doi.org/10.3329/bjmb.v5i1.13428

Razak, D. L. A., Ghani, A. A., Lazim, M. I. M., Khulidin, K. A., Shahidi, F., & Ismail, A. (2024). Schizophyllum commune (Fries) Mushroom: A Review on Its Nutritional Components, Antioxidative and Anti-inflammatory Properties. *Current Opinion in Food Science*, 101129. https://doi.org/10.1016/j.cofs.2024.101129

Ray, P., Kundu, S., & Paul, D. (2024). Exploring the Therapeutic Properties of Chinese Mushrooms with a Focus on their Anti-Cancer Effects: A Systemic review. *Pharmacological Research - Modern Chinese Medicine*, 100433. https://doi.org/10.1016/j.prmcm.2024.100433

Real Food Encyclopedia - Mushrooms - FoodPrint. (2024, March 4). FoodPrint. https://foodprint.org/real-food/mushrooms/

Robinson, N. (2024, July 16). Exploring the role of mushrooms throughout history. R&R Cultivation. https://rrcultivation.com/blogs/mn/exploring-the-role-of-mushrooms-throug hout-history

Rokaya, M. B., Parajuli, B., Bhatta, K. P., & Timsina, B. (2019). Neopicrorhiza scrophulariiflora (Pennell) Hong: A comprehensive review of its traditional uses, phytochemistry, Ethnopharmacology, pharmacology and safety. *Journal of 247*, 112250. https://doi.org/10.1016/j.jep.2019.112250

Rosnan, N.D., Chuen, N.L. and Ngadin, A.A. (2019). First record of in vitrogrowth evaluation of wild mushroom,

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Schizophyllum communefrom Pulau Kapas in Malaysia. *Asian Journal of Agriculture and Biology*. 7(4): 602-609 https://www.cabidigitallibrary.org/doi/pdf/10.5555/20203166791

Saetang, N., Ramaraj, R., Praphruet, R., & Unpaprom, Y. (2023). Unlocking the benefits of split gill mushroom: Chemical analysis and prebiotic properties of schizophyllan extract. *International Journal of Biological Macromolecules*, 252, 126544. https://doi.org/10.1016/j.ijbiomac.2023.126544

Ślusarczyk, J., Adamska, E., & Czerwik-Marcinkowska, J. (2021). Fungi and algae as sources of medicinal and other biologically active compounds: a review. *Nutrients*, 13(9), 3178. https://doi.org/10.3390/nu13093178

Shady, N. H., Sobhy, S. K., Mostafa, Y. A., Yahia, R., Glaeser, S. P., Kämpfer, P., El-Katatny, M. H., & Abdelmohsen, U. R. (2023). Phytochemical analysis and anti-infective potential of fungal endophytes isolated from Nigella sativa seeds. *BMC Microbiology*, 23(1). https://doi.org/10.1186/s12866-023-03085-4

Sharma, A., Rashid, M., Chauhan, P., Kaur, S., & Kaur, A. (2024). In vitro antibacterial and anti-biofilm potential of an endophytic Schizophyllum commune. *AMB Express*, 14(1). https://doi.org/10.1186/s13568-024-01663-x

Sharma, B. R., Kumar, V., Gat, Y., Kumar, N., Parashar, A., & Pinakin, D. J. (2018). Microbial maceration: a sustainable approach for phytochemical extraction. *3 Biotech*, 8(9). https://doi.org/10.1007/s13205-018-1423-8

Shevchuk, Y., Kuypers, K., & Janssens, G. E. (2023). Fungi as a source of bioactive molecules for the development of longevity medicines. *Ageing Research Reviews*, 87, 101929. https://doi.org/10.1016/j.arr.2023.101929

Singer Instruments. (n.d.). Zone of inhibition explained. https://www.singerinstruments.com/resource/zone-of-inhibition-explained/

Stajić, M., Galić, M., & Ćilerdžić, J. (2023). Mushrooms – from traditional remedies to the modern therapeutics. In *Mushrooms with Therapeutic Potentials* (pp. 147–188). https://doi.org/10.1007/978-981-19-9550-7_5

Studio Animato. (n.d.). Shiitake. MycoMedica. https://www.mycomedica.eu/shiitake.html

Taylor, T. A., & Unakal, C. G. (2023, July 17). Staphylococcus aureus Infection. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK441868/

Tetteh, J. N., Matthäus, F., & Hernandez-Vargas, E. A. (2020). A survey of within-host and between-hosts modelling for antibiotic resistance. *Biosystems*, 196, 104182. https://doi.org/10.1016/j.biosystems.2020.104182

Tripathi, N., & Sapra, A. (2023). Gram staining. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK562156/

Antibiotic Research UK. (2023). Types of bacteria | Gram-Positive and Gram-Negative bacteria. https://www.antibioticresearch.org.uk/about-antibiotic-resistance/bacterial-infections/types-of-bacteria/?fbclid=IwZXh0bgNhZW0CMTEAAR3_wKXWf 1XZ0EuWjaH6aBSWIXEdEdhCzZel0YY0Ii8GGpV7yh8Ck1YV9gk_aem_g lrC1bQ7asZnDp8MvVyCrw

Ugbogu, E. A., Akubugwo, I. E., Ude, V. C., Gilbert, J., & Ekeanyanwu, B. (2019b). Toxicological Evaluation of Phytochemical Characterized Aqueous Extract of Wild Dried Lentinus squarrosulus (Mont.) Mushroom in Rats. *Toxicological Research*, 35(2), 181–190. https://doi.org/10.5487/tr.2019.35.2.181

Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International journal of microbiology*, 2015, 376387. https://doi.org/10.1155/2015/376387

Venkatesan, T., Choi, Y., & Kim, Y. (2019). Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of Pinus densiflora Bark Extract. *BioMed Research International*, 2019, 1–14. https://doi.org/10.1155/2019/3520675

Victoria Department of Health and Human Services [DHH]. (n.d.). Streptococcal infection - group A. Better Health Channel. https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/strepto coccal-infection-group-a#

Vieira, V. V., Teixeira, L. M., Zahner, V., Momen, H., Facklam, R. R., Steigerwalt, A. G., Brenner, D. J., & Castro, A. C. D. (1998). Genetic relationships among the different phenotypes of Streptococcus dysgalactiae strains. *International Journal of Systematic Bacteriology*, 48(4), 1231–1243. https://doi.org/10.1099/00207713-48-4-1231

Welle, D. (2022, October 17). A brief cultural history of the mushroom. dw.com. https://www.dw.com/en/a-brief-cultural-history-of-the-mushroom/a-634613 80

Wongaem, A., Reamtong, O., Srimongkol, P., Sangtanoo, P., Saisavoey, T., & Karnchanatat, A. (2020). Antioxidant properties of peptides obtained from the split gill mushroom (Schizophyllum commune). *Journal of Food Science and Technology*, 58(2), 680–691. https://doi.org/10.1007/s13197-020-04582-4\

Wu, P., Zhang, Z., Luo, Y., Bai, Y., & Fan, J. (2022). Bioremediation of phenolic pollutants by algae - current status and challenges. *Bioresource Technology*, 350, 126930. https://doi.org/10.1016/j.biortech.2022.126930

BTTHS Journal of Students' Projects in Advancing Research Knowledge (S.P.A.R.K)

Wzorek-Łyczko, K., Woźniak, W., Piwowarczyk, A., & Kuchar, E. (2023). The anti-infective effect of β-glucans in children. *International Journal for Vitamin and Nutritional Research*, 94(3-4), 296-307. https://doi.org/10.1024/0300-9831/a000793

Yim, H.S., Chye, F.Y., Heng, P.Y. and Ho, C.W. (2011) Oxidative Stability of Sunflower Oil Supplemented with Medicinal Split Gill Mushroom, Schizophyllum commune Fr.: Fr. Extract during Accelerated Storage. *International Journal of Medicinal Mushrooms*, 13, 357-368. https://doi.org/10.1615/IntJMedMushr.v13.i4.60

Yu, Q., & Fan, L. (2021). Improving the bioactive ingredients and functions of asparagus from efficient to emerging processing technologies: A review. *Food Chemistry*, 358, 129903. https://doi.org/10.1016/j.foodchem.2021.129903

Bacolod Tay Tung High School, Inc.
P. Hernaez Street, Bacolod City, Negros Occidental, Philippines, 6100
Tel. No.: (034) 433 2702
Website: www.btths.edu.ph