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Bacteriostatic Effect of Fresh Coconut Husk Smoke Residue on Inoculated Glass Slides Contaminated with *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

The study determines the potential of coconut husk smoke residue as a passive bacteriostatic agent against *Staphylococcus aureus* and *Escherichia coli*. This study utilized an experimental-comparative design to evaluate the antibacterial activity of smoke residue obtained from fresh, mature coconut husks. Microscope glass slides were exposed to the smoke residue and inoculated with bacterial suspensions, with comparisons made among treated, untreated, and positive control groups based on colony-forming unit (CFU/mL) counts. Due to non-normal data distribution, the Kruskal-Wallis H test and Mann-Whitney U test with Bonferroni correction were applied. Findings revealed a statistically significant reduction in CFU/mL of *Staphylococcus aureus* on treated surfaces ($p = .002$), indicating a bacteriostatic effect. However, no significant difference was observed for *Escherichia coli* ($p = .982$). These results suggest that while coconut husk smoke residue exhibits antibacterial potential, its effect remains inconsistent and insufficient as a reliable alternative to conventional disinfection methods in practical applications. Further studies are recommended to standardize experimental conditions, improve detection methods, and identify the active compounds responsible for its antimicrobial activity and overall efficacy in different microbial environments tested. Additionally, future research may explore variations in exposure time, concentration of smoke residue, and environmental factors that could influence antibacterial performance and reproducibility of results.

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1.0 INTRODUCTION

Cocos nucifera, commonly known as coconut, is a widely cultivated tropical plant whose husk is often considered an agricultural by-product. Coconut husk is primarily composed of lignin, cellulose, and hemicellulose and contains phenolic compounds known for their potential antimicrobial and antioxidant properties.[1] Previous studies have identified coconut husk as a rich source of bioactive compounds, making it a promising material for various biological and environmental applications. When subjected to combustion or pyrolysis, coconut husk releases phenols, acids, and volatile organic compounds that are associated with antimicrobial activity against microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.[2] Traditional disinfection methods using smoke have long been practiced in various cultures. In Kenya, pastoralist communities use smoke derived from burning plant materials to disinfect milk containers.[3] This practice has been examined in scientific studies, which suggest that smoke treatment contributes to reduced microbial presence on surfaces. Similarly, research on coconut-derived liquid smoke has explored its antimicrobial properties and its potential application in food preservation. Coconut-based smoke

products have been investigated, highlighting the presence of phenolic and flavonoid compounds associated with antimicrobial activity.[2,4,5]

In addition, combustion-derived materials have also been explored for their antimicrobial potential. Carbon-based particles obtained from soot have been investigated,[6] while compounds produced through the pyrolysis of coconut fiber have also been studied.[7] These studies emphasize the presence of bioactive compounds formed during combustion processes. Furthermore, the antimicrobial mechanisms of phenolic compounds have been studied, including their ability to interact with microbial cell structures and biological processes.[8]

Despite these existing studies, most research has focused on processed forms such as liquid smoke, purified extracts, or chemically modified compounds. Limited attention has been given to the direct use of raw smoke residue as a surface disinfectant. This gap is particularly relevant in the Philippines, where microbial contamination in food products remains a concern, including the presence of *Escherichia coli* and *Staphylococcus aureus* in commonly consumed goods. These concerns highlight the need for accessible, cost-effective, and sustainable sanitation alternatives.

In recent years, there has been increasing interest in developing environmentally friendly substitutes for conventional disinfectants. However, no prior research has specifically investigated the use of coconut husk smoke residue in its unprocessed form as a surface-active antimicrobial agent. In this study, *Staphylococcus aureus* and *Escherichia coli* are used as test organisms to evaluate the potential of coconut husk smoke deposits as a passive disinfectant layer. The purpose of this study is to assess its effectiveness as a natural, low-cost, and sustainable alternative for microbial control.

This study is anchored on several theoretical frameworks. The Germ Theory of Disease, established by scientists such as Louis Pasteur, Robert Koch, and Joseph Lister, explains that microorganisms are the primary cause of infections and supports the importance of disinfection in preventing microbial transmission.[9] The Circular Economy Theory emphasizes the reuse of waste materials, highlighting coconut husk as a valuable resource rather than discarded waste.[10] Additionally, the Chemiosmotic Theory of Antibacterial Action of Phenolics, proposed by Peter Mitchell, explains how phenolic compounds may disrupt bacterial cell membranes and interfere with proton gradients essential for ATP production,[11,12] thereby inhibiting bacterial growth. These theories collectively support the potential of coconut husk smoke residue as a natural and sustainable antimicrobial agent.

This study aimed to evaluate the bacteriostatic activity of fresh coconut husk smoke residue on glass slide surfaces contaminated with *Staphylococcus aureus* and *Escherichia coli*. Specifically, this study sought to address the following objectives:

1. To evaluate the bacteriostatic activity of fresh coconut husk smoke residue against the following two different bacterial species:

1.1. *Staphylococcus aureus*; and

1.2. *Escherichia coli*

2. To compare the bacteriostatic activity of coconut husk smoke residue between treated surfaces versus untreated surfaces against *S. aureus* and *E. coli*, in terms of Colony-forming unit (CFU) count

Hypothesis of the Study

At the 0.05 level of significance, the following are tested:

H_{01}: Fresh mature coconut husk smoke residue does not have any bacteriostatic activity against two different bacterial species.

1.1 *Staphylococcus aureus*

1.2 *Escherichia coli*

H_{02}: There is no bacteriostatic activity of fresh coconut husk smoke residue between treated surfaces versus untreated surfaces against *S. aureus* and *E. coli*, in terms of Colony-forming unit (CFU) count (CFU/mL)

H_{A1}: Fresh mature coconut husk smoke residue has a bacteriostatic activity against two different bacterial species.

1.1 *Staphylococcus aureus*

1.2 *Escherichia coli*

H_{A2}: There is a bacteriostatic activity of fresh coconut husk smoke residue between treated surfaces versus untreated surfaces against *S. aureus* and *E. coli*, in terms of Colony-forming unit (CFU) count (CFU/mL)

2.0 METHODOLOGY

2.1 Research Design, Environment, Instruments

The study utilized an experimental research design in the evaluation of the bacteriostatic activity of coconut husk smoke residue against *Staphylococcus aureus* and *Escherichia coli*. It is also used to find the differences between the treated slides and untreated slides in terms of the CFU count and the residual activity after immediate post-treatment.

INPUT	PROCESS	OUTPUT
Staphylococcus aureus *Escherichia coli* Coconut husk residue Sterile microscope slide Sterile petri dish Sterile test tubes Sterile glass container Incubator	Experimental, Comparative Design Research environment Research instrument Research procedure Ethical consideration Data Analysis	Bacteriostatic activity of coconut husk residue against *S. aureus* and *E. coli* Treated vs. Untreated microscope slides in terms of CFU count

Figure 1. Research Flow Process

The experiment was performed inside the General Chemistry and Microbiology-Parasitology Laboratory on the 8th floor, Room 811 of the University of Cebu - Banilad Campus, with controlled laboratory environmental conditions using the fume hood and biosafety cabinet (BSC) to determine the bacteriostatic activity of coconut husk residue against *Staphylococcus aureus* and *Escherichia coli*. A colony counter is a device that is often used to count bacterial or other microorganism colonies on a petri dish that contains a gelled growth medium. It is divided into two types: manual colony counter and automatic colony counter. Both can be used to determine the CFU, or how many bacteria are present in a given sample. The Quebec Darkfield Colony Counter is a manual colony counter that is available in the laboratory where the study was performed. With it, the researchers can gather data and follow the objectives of the study.

2.2 Research Procedure and Analysis

Fresh mature coconuts were obtained from locally available dwarf-type coconut trees in Kalawisan, Lapu-Lapu City. Before experimenting, the coconut was brought to the Philippine Coconut Authority Regional Office VII in Mandaue City, Cebu, for validation of its species to confirm if it is indeed *Cocos nucifera* L. Once confirmed, the coconut husks were separated from the shell.

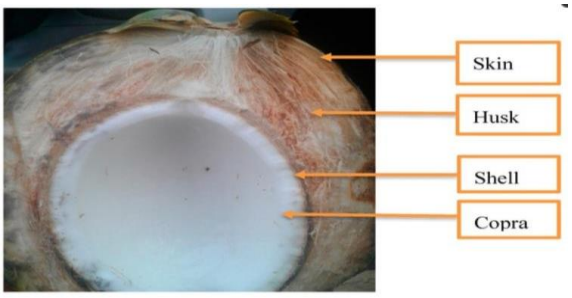

Figure 2. Half-view of whole coconut showing the husk, skin, shell, and copra.[13]

In accordance with the objectives of the study, the researchers have formed the following groups:

1.1 - Treated, *Staphylococcus aureus*
1.2 - Treated, *Escherichia coli*
2.1 - Untreated, *Staphylococcus aureus*
2.2 - Untreated, *Escherichia coli*
PC - Positive Control (70% Ethanol) NC - Negative Control (Smoke control)

Each group has 15 slides, except for the controls. The sample size was recommended by the researchers' statistician.

The materials used in the setup were microscope glass slides as the test surface and petri dishes to hold the glass slides; specifically, four (4) glass slides were securely affixed using double-sided tape in one (1) 150x15mm petri dish side by side with a 5mm distance between each other. An exception to the last sentence would be that the last petri dish of each experimental group contains only three slides per petri dish, as each experimental group has been recommended to have 15 samples. To standardize the inoculation area, each microscope slide was marked with a 2×2 cm square by placing a piece of masking tape at the back of the slide. Still at the back of the slide, each slide was labeled with different numbers in order not to retest the same slide repeatedly. Furthermore, each petri dish was labeled on its base with the group to which they belonged and the bacterial species to be inoculated on the slides adhered to them.

A glass container was also used to provide a controlled environment. This container is critical to the setup as it maintains consistent internal conditions such as limited airflow, smoke concentration, and minimal external concentration. Additionally, a tin can was used to contain the coconut husk while being ignited. In order to reduce contamination when putting items inside

when needed, tongs were also utilized. For the colony counting of bacterial growth, standard-size (100x15mm) petri dishes were used. All materials were thoroughly sterilized via ultraviolet (UV) light exposure in the Biosafety Cabinet (BSC) of the Microbiology Laboratory within 8 feet of the UV source for 30 minutes for efficient inactivation of bacteria.[14] Microcentrifuge tubes and test tubes used for bacterial suspension and serial dilutions were autoclaved prior to use. These were done to ensure complete sterility and ensure that the bacterial growth in the study is not due to material contamination.

Nutrient agar served as the general-purpose culture medium for growing non-fastidious bacteria in this study. It was chosen because it can support a variety of microbial species, like *Escherichia coli* and *Staphylococcus aureus*, without needing extra growth factors. Preparation followed the manufacturer's instructions on the bottle label. For each liter of distilled water, 28 grams of nutrient agar powder was added. The mixture was heated while stirring continuously until it dissolved completely and became clear. After boiling, the solution was sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Once it cooled to about 45–50 °C, the medium was poured into sterile Petri dishes and left to solidify.

The whole setup occurred inside a fume hood located in the General Chemistry Laboratory of the University of Cebu - Banilad. This container functions as the smoke chamber. It provides the enclosed, sterile environment necessary to trap the coconut husk smoke and ensure consistent exposure of the glass slides to antimicrobial compounds. A tin can was prepared to hold the coconut husk. The coconut husks were then ignited carefully to generate smoke while avoiding the production of large flames. Once the smoke was under control, the tin cans containing the smoking coconut husks were placed carefully inside the glass container using sterilized tongs, ensuring the cans did not come into any contact with the internal surfaces of the glass containers, minimizing potential contamination, and preserving the sterility of the setup. If large flames began to form during the process, the coconut husks in the tin can were replaced with new ones, and the smoke was carefully regulated again. The petri dish, taped with glass slides that are to be inoculated with the same bacteria, was held inverted above the opening lid of the glass container and gently repositioned while maintaining the standard 15 cm distance positioned to follow the smoke flow, ensuring optimal exposure.[15] The microscope slides were ensured not to touch the flames and were 15 cm above the smoke. Once a visible, even smoke residue layer formed after 3 minutes, the petri dish with the treated microscope slides was put aside in the Biosafety cabinet to cool down with the petri dish cover half-closed. In contrast, untreated microscope slides were not exposed to smoke or any type of disinfectant and were simply kept untouched in the biosafety cabinet until bacterial inoculation. Control microscope slides were also prepared: positive control and negative control. The positive control microscope slide against *S. aureus* and *E. coli* was treated with 70% ethanol, and the negative control microscope slide was exposed to the coconut husk smoke, but with no inoculation. The smoke control microscope slide serves as proof of potential bacterial contamination from the smoke setup.

A 0.5 McFarland turbidity standard, which is about 1.5×10^8CFU/mL, was made by mixing 0.5 mL of 1% barium chloride with 9.95 mL of 1% sulfuric acid.[16] The turbidity of the standard was verified by measuring its absorbance at 625 nm with a spectrophotometer that had a 1 cm light path. Acceptable absorbance values were between 0.08 and 0.10.

Bacterial suspensions of *Staphylococcus aureus* and *Escherichia coli* were prepared aseptically by transferring a loopful of fresh colonies into 10 mL of sterile normal saline solution (NSS). The suspension was visually compared to the 0.5 McFarland standard. An aliquot was then transferred into a clean cuvette and read at 625 nm. If the absorbance fell outside the acceptable range, adjustments were made: sterile NSS was added if the absorbance was too high, or additional bacterial colonies were incorporated if it was too low. After each measurement, the used cuvette was decontaminated with 10% Lysol, and a fresh cuvette was used for subsequent readings. Gloves were changed between handling to maintain aseptic technique.

After the coconut husk smoke-treated microscope slides had cooled down, they were immediately inoculated with their respective bacteria, *Staphylococcus aureus* or *Escherichia coli*, ensuring that in one (1) petri dish with multiple slides, all slides would be inoculated with the same bacterial species. Using a sterile swab, streak a 2×2 cm area on each microscope slide with their respective bacterial suspensions. This specific measurement allows the standardization of the inoculation. After streaking, the slides are allowed to air-dry for 5-10 minutes at room temperature. The same procedures were done on the untreated slides and the positive control, with the positive control being inoculated with the bacteria while the 70% ethanol was still wet. To prevent condensation during incubation, Petri dishes containing the smoked and inoculated microscope slides were left half-covered during the cooling phase. This allowed residual heat to dissipate while maintaining sterility. After the slides reached ambient temperature, the lids were sealed, and the dishes were incubated at 37°C for 18–24 hours.[17]

After incubation, a sterile swab pre-moistened with saline was used to collect bacteria for each slide, making sure to cover the 2x2 mm square area thoroughly to ensure maximum bacterial recovery. The swab was vigorously mixed to dislodge and suspend the bacteria in 1 mL of sterile saline solution, and using the suspension, a serial dilution was performed.

For the serial dilution, the researchers prepared three tubes, each containing 900 µL of sterile saline. From the original 1 mL saline mixture, which contained the bacteria, the researchers pipetted 100 µL and transferred it into the first tube, creating a 1:10 dilution. The solution was mixed thoroughly to ensure a uniform bacterial suspension.

Next, from the 1:10 dilution tube, the researchers took another 100 µL and transferred it to the second tube to create a 1:100 dilution. The researchers mixed it well again. The process was repeated by pipetting 100 µL from the 1:100 dilution and adding it to the third tube to create a final 1:1,000 dilution.

After preparing the serial dilutions, the researchers plated 100 µL from each of the three dilution tubes—1:10, 1:100, and 1:1,000—onto separate nutrient agar plates. For plating, 100 µL was pipetted onto the surface of each plate and spread evenly using a plate spreader to allow for isolated colony growth.[18] Plates were incubated at 37°C for 18–24 hours, after which visible colonies were counted using standard microbiological techniques.

Data Analysis

After counting the colonies for each plate, the researchers used the following formula to calculate the CFU/mL of each plate:

$$\text{CFU/mL} = \frac{counted\ colonies \times dilution\ factor}{vol.\ of\ specimens\ used}$$

Data gathered has been verified by a licensed microbiologist to ensure that the data tabulated were correct. The data were treated using the Kruskal-Wallis H-test with a significance level set at $p < 0.05$, followed by the Mann-Whitney U-test if there is a significance after treatment of data with the Kruskal-Wallis H-test. Analysis of data is conducted with the help of a statistician to ensure that the right treatment of data is done. The statistical instrument used was Statistical Package for the Social Sciences 25 (SPSS25) and Minitab18.

3.0 RESULTS AND DISCUSSION

This chapter shows the bacteriostatic activity of *Cocos nucifera* L. husk smoke residue against *Staphylococcus aureus* and *Escherichia coli*, and compares its effect against untreated slides. The researchers collected the data in this study directly from their experiments. The findings are presented in comprehensive tables that illustrate the quantitative colony-forming unit (CFU) count using the spread plate method.

3.1 CFU/ mL of Treated and Untreated Slides

To evaluate the bacteriostatic effect of fresh coconut husk smoke residue on glass slides contaminated with *Staphylococcus aureus* and *Escherichia coli*, the colonies formed by group 1.1, 1.2, 2.1, and 2.2 were counted using a Quebec Darkfield Colony Counter. The resulting data addressed the two (2) objectives of the study: (1) To evaluate the bacteriostatic activity of fresh coconut husk smoke residue against *Staphylococcus aureus* and *Escherichia coli*, and (2) To compare the bacteriostatic activity of coconut husk smoke residue between treated surfaces versus untreated surfaces against *S. aureus* and *E. coli*, in terms of: Colony-forming unit (CFU) count, and Residual activity after immediate post-treatment of coconut husk smoke residue.

3.2 Bacteriostatic effect of treatment to *S. aureus* and *E. coli*

The assumptions for performing ANOVA or t-test include normal distribution of data, homogeneity of variance among groups, independence of observations within each group, and random sampling of observations.[19]

Table 1. Preliminary Testing of Assumptions

		Kolmogorov-Smirnov[a]		Shapiro-Wilk	
	Treatment	df	Sig.	df	Sig.
CFU/mL *S. aureus*	Positive	3		3	
	Treated	44	.000	44	.000
	Untreated	44	.000	44	.000
CFU/mL *E. coli*	Positive	3		3	
	Treated	44	.000	44	.000
	Untreated	44	.000	44	.000

Table 1 shows whether the assumptions have been met to perform ANOVA or a t-test. Using the Shapiro-Wilk Normality test, one can identify whether there is significant deviation from the normal distribution if the P-value is less than the 0.05 level of significance or if there is an absence of results, which is indicated in Table 2.0, where the level of significance is absent. It indicates that the datasets are not normally distributed, which results in the treatment of data using the Kruskal-Wallis H-test and a Post Hoc Test using the Mann-Whitney U-test with a Bonferroni Correction of the Significance Level to 0.025. With those statistical tools, the statistician used Statistical Package for the Social Sciences 25 (SPSS25) and Minitab18 for a clean treatment of data.

Table 2. Kruskal-Wallis Test: *S. aureus* and *E. coli*

	N	Median	Mean Rank	χ^2	p-value
Treatment (S. aureus)					
Positive control	3	0	35		
Treated	45	0	40.7	10.43	0.005
Untreated	45	0	54.1		
Treatment (*E. coli*)					
Positive control	3	0	37		
Treated	45	0	47.3	0.83	0.661
Untreated	45	0	47.4		

Kruskal-Wallis test indicated a significant difference in *S. aureus* CFU/mL values among the three groups ($\chi^2 = 10.43$, $p = 0.005$). This suggests that at least one group differed significantly in bacterial count. The Kruskal–Wallis H test was also conducted to compare *E. coli* CFU/mL across the same three treatment groups. Results showed no significant difference among them ($\chi^2 = 0.83$, $p = 0.661$), indicating that the treatments did not alter *E. coli* bacterial counts significantly. To identify and confirm what groups significantly differed, Mann–Whitney U tests were performed between pairs of groups.

Table 3. Mann-Whitney U Test: *S. aureus* and *E. coli*

	U-Value	p-Value	Interpretation
Comparison (*S. aureus*)			
Treated vs. Untreated	720.00	0.002	Significant difference
Treated vs. Positive Control	58.50	0.504	Not significant
Untreated vs. Positive Control	40.50	0.185	Not significant
Comparison (*E. coli*)			
Treated vs. Untreated	1010.500	0.982	Not significant
Treated vs. Positive Control	52.500	0.368	Not significant
Untreated vs. Positive Control	52.500	0.367	Not significant

The treated group was effective in significantly reducing *S. aureus* CFU/mL compared to the untreated group ($p = 0.002$). However, there was no significant difference ($p = 0.504$) between treated and positive control samples, implying that the treatment's bacteriostatic activity was comparable to that of the control.

The treated group did not produce any significant bacteriostatic effect on *E. coli*. The following groups under *E. coli*: Treated vs. Untreated ($p = 0.982$), Treated vs. Positive Control($p = 0.368$), and Untreated vs. Positive Control ($p = 0.367$), showed nearly identical mean ranks, indicating there were no significant differences between all of them, and the treatment's bacteriostatic activity was ineffective against *E. coli*.

Previous studies have documented the antimicrobial efficacy of coconut-derived materials. Coconut husk extract has been reported to reduce microbial counts and delay spoilage, with observed reductions in bacterial growth in treated samples, supporting its potential antimicrobial application.[20]

Phenolic compounds have been investigated for their antimicrobial activity against pathogenic multidrug-resistant Staphylococcus aureus, particularly strains obtained from food sources. These compounds include thymol, eugenol, carvacrol, protocatechuic acid, and hydroquinone, which were evaluated for their inhibitory effects on S. aureus isolates. Among the tested compounds, hydroquinone exhibited notable antibacterial activity, demonstrating a comparatively higher minimum inhibitory concentration (MIC) value with an average of 54 µg/mL when tested against different strains of S. aureus.[21]

Coconut husk extract has also been reported to exhibit weaker antimicrobial effect against Gram-negative bacteria compared to Gram-positive bacteria. Reductions in bacterial load were observed in treated fish samples, with stronger inhibitory effects noted

particularly against *Staphylococcus aureus*.[20]

These findings are consistent with the current study's results, which showed no statistically significant reduction in *E. coli* CFU/mL on treated slides compared to untreated or positive control groups. The results of this study align with the broader literature, suggesting that natural treatments, such as coconut husk smoke residue, may be less effective against Gram-negative bacteria, like *E. coli*.[8] The absence of statistically significant differences across all comparisons for *E. coli* (treated vs. untreated, treated vs. positive control, and untreated vs. positive control) may be due to the inherent structural defense mechanisms of *E. coli*.

4.0 CONCLUSION

The researchers concluded that statistical analysis revealed a significant difference in the bacteriostatic activity of fresh coconut husk smoke residue among the tested groups for *Staphylococcus aureus*, while no significant effect was observed for *Escherichia coli*. The Kruskal–Wallis test indicated a significant variation in CFU/mL for *S. aureus*, and post hoc analysis showed that the Treated group had significantly lower bacterial counts compared to the Untreated group, suggesting that the treatment was effective in reducing *S. aureus* growth; however, no significant differences were found between the Treated and Positive Control groups, as well as between the Untreated and Positive Control groups, indicating comparable levels of bacterial growth among these conditions. In contrast, both the Kruskal–Wallis and Mann–Whitney U-test results for *E. coli* showed no significant differences among all groups, confirming that the treatment did not significantly affect its CFU/mL. Overall, the findings suggest that fresh coconut husk smoke residue exhibits selective antimicrobial activity, being effective against *Staphylococcus aureus* but ineffective against *Escherichia coli* under the conditions tested.

AUTHORS CONTRIBUTORS

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