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Antibacterial Solution Using *Cinnamomum verum* (Cinnamon) and *Cymbopogon citratus* (Lemongrass) Essential Oils with Hyrdogen Peroxide Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Amidst the pandemic, millions of impoverished Filipinos lack sufficient access to sanitation services that protect them from bacterial infections. As such, this study endeavored to formulate a disinfectant spray of hydrogen peroxide and varying concentrations of lemongrass (Cymbopogon citratus) and cinnamon (Cinnamomum verum) essential oils to produce a maximally effective solution. Due to their natural origin, these essential oils were chosen for their extensive antibacterial properties, affordable price, and low toxicity levels. Six disinfectant spray solutions containing different concentrations of either essential oil were tested against Staphylococcus aureus and Escherichia coli bacteria through the agar disk diffusion method. After analyzing the data using mean, standard deviation, and MANOVA, it was found that including cinnamon and lemongrass essential oil had no statistically significant effect on the antibacterial activity of the hydrogen peroxide solution, regardless of the concentration of essential oil used, $p > 0.05$. However, the lemongrass samples were slightly more effective than the cinnamon solutions. Thus, future researchers are encouraged to investigate other components that can potentially increase the antibacterial activity of a disinfectant spray.

Keywords: *Cinnamomum verum; Cymbopogon citratus*; antibacterial activity; disinfectant; agar disk diffusion

INTRODUCTION

Although limited and insufficient access to sanitation materials compromise the inherent right to proper health, antibacterial solutions are imperative to ensure effective sanitation for the population. Communities across the globe struggle with the availability and accessibility of sanitation services. Thus, the 2030 Agenda for Sustainable Development consists of seventeen Sustainable Development Goals (SDGs), which provide a holistic approach to achieve sustainable development for all by the year 2030. Globally, the sixth SDG, Clean Water and Sanitation, aims to "ensure availability and sustainable management of water and sanitation for all" (United Nations Development Programme, 2015). More than this, the pandemic has drastically increased the need for new and innovative sanitation materials. However, poverty and shortages of supply make these sanitation needs unattainable. Additionally, the toxicity of alcohol, a component of most disinfectant sprays, is another concern that has detrimental effects on the consumer's health (Mahmood et al., 2020). As such, researchers suggested exploring new formulations for sanitation products in the hope that these will aid in increasing the supply and promoting public awareness.

Instead of synthetic and chemical compounds that may impose toxic risks, natural materials with antimicrobial properties also prove to be a suitable alternative, with a significantly lower price that can allow underprivileged communities to purchase them. Thus, a much safer and more accessible option is using essential oils, though there have been numerous debates on using them due to their supposed inefficacy. However, previous findings revealed essential oils to possess an effective antibacterial activity and low toxicity levels due to their natural origin (Cattelan et al., 2013; Swamy et al., 2016; Winska et al., 2019). Among these essential oils, cinnamon and lemongrass possess extensive antibacterial properties against many bacteria. Research conducted by Raeisi et al. (2015) pointed out that cinnamon is a fragrant plant that can be used to flavor and preserve food. Additionally, it has components that can fight against *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Bacillus cereus,* and *Salmonella typhimurium*. On the other hand, lemongrass essential oils and methanol extracts were combined to create lemongrass methanol in the research conducted by Jafari et al. (2012). This was done to measure activities involving antimicrobials through agar well diffusion and dilution. It was later revealed that lemongrass and its essential oils displayed more antibacterial activity against gram-positive bacteria. Lastly, various concentrations of these two essential oils were further investigated in this research to discover the concentration needed to inhibit the bacterial growth of prominent pathogens.

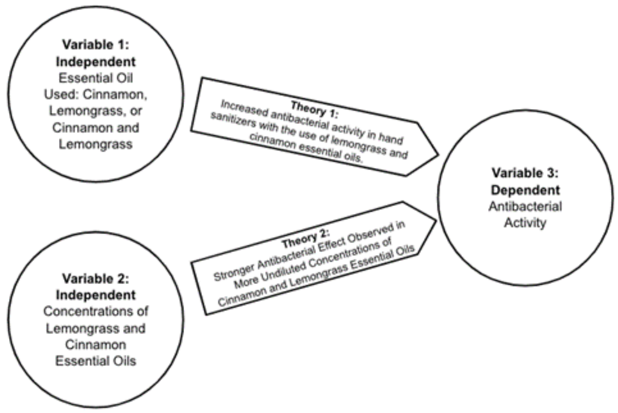
In addition to concentrations of essential oils, hydrogen peroxide was also used as a major component of the disinfectant spray. Hydrogen peroxide has a strong antibacterial activity; thus, a disinfectant spray was made primarily from this material to replace alcohol to alleviate the issues surrounding the latter's toxicity. Cadnum et al. (2015) assessed the efficacy of a hydrogen peroxide spray in reducing the contamination of soft surfaces in hospitals. An experiment on two hospitals was performed, with hydrogen peroxide tested on 200 soft surfaces in one hospital and 233 soft surfaces in the other. It was revealed that the hydrogen peroxide spray possesses an effective antibacterial activity that can inhibit bacterial growth on hospital surfaces. Likewise, the efficacy of a hydrogen peroxide-based disinfectant was investigated to evaluate its antibacterial properties. The results found that the hydrogen peroxide-based disinfectant had an efficacy of 100% at a concentration of 2% with a short exposure time (Medina-Cordoba et al., 2018).

Furthermore, antibacterial activity is described as killing or inhibiting microbes and bacteria (Song et al., 2019). Any material used in the sanitation process must possess a strong antibacterial activity that effectively decontaminates surfaces and reduces disease-causing pathogens and bacteria. The preceding variables are important factors that can significantly affect the antibacterial activity of the formulation; hence the antibacterial activity is considered a dependent variable. Though other factors can influencemantibacterial activity, they are not the focal points of this research.

In this paper, it is theorized that the antibacterial activity in sanitizing materials can be increased using essential oils, specifically lemongrass and cinnamon essential oils. The three variables in this research, namely (1) the essential oil used, whether cinnamon or lemongrass; (2) the concentrations of the essential oil; and (3) the antibacterial activity, are supported by numerous studies. Two theories also outline the relationship between these three variables. The first is that there is increased antibacterial activity in hand sanitizers that incorporate the use of essential oils, and the second is that there is a stronger antibacterial effect observed in more undiluted concentrations of cinnamon and lemongrass essential oils. These relationships between the variables are justified, as the previous studies show the essential oils' effectiveness when applied individually. This may provide insights into how the dependent variable might change when the antibacterial activity of the cinnamon and lemongrass essential oils added to the hydrogen peroxide are tested. Moreover, numerous studies also attest to increased antibacterial activity in solutions that incorporate higher concentrations of essential oils.

Figure 1

Conceptual Framework



However, it has not been mentioned whether these materials would show any change in antimicrobial activity if combined with hydrogen peroxide, as a combination could affect their antibacterial activity. The concentration of the essential oil is also important, as the oils may present a difference in their antibacterial activity depending on their concentration. As a result, relationships between the variables can be established, as a change in the concentration (variable 2) or a change in the composition (variable 1) of the disinfectant could affect its antibacterial activity (variable 3).

Statement of the Problem

This research aims to develop a disinfectant spray solution using essential oils, namely *Cinnamomum verum* (Cinnamon) and *Cymbopogon citratus* (Lemongrass), alongside hydrogen peroxide to heighten its antibacterial effects. Furthermore, it seeks to answer the following research questions:

1. How does the essential oil (cinnamon or lemongrass) affect the antibacterial activity of the disinfectant spray formulation?
2. How do essential oil concentrations influence the antibacterial activity of the disinfectant spray formulation?

Scope and Limitations

Numerous limitations that must be considered due to the current pandemic. This research only involved cinnamon and lemongrass essential oils due to their effective antibacterial properties, as proven in previous studies. However, the researchers could not extract these essential oils manually due to the constraints of the pandemic. Moreover, the solutions' antibacterial activity will be the determining factor in assessing whether or not they can be used as a disinfectant spray since other factors, such as compatibility with all surfaces, require additional resources and further research. Lastly, the solutions will only be tested against *Staphylococcus aureus* and *Escherichia coli* since these bacteria are the conventional ones used for investigating antibacterial activity, and they represent gram-positive and gram-negative bacteria, respectively. However, the antibacterial solutions' effects on these bacteria do not indicate their effects on all types of bacteria.

METHODOLOGY

Research Design

The study used an experimental approach, specifically the factorial design, to collect the necessary data and establish conclusions. The factorial design of the study allowed the researchers to analyze the interactions between all variables involved. The 2x3 factorial design is presented in Table 1 below.

Table 1

Factorial Research Design

2x3 Design		**Essential Oil Used**	
		Cinnamon (V1A)	Lemongrass (V1B)
Concentration	1% Concentration (V2A)	V1A V2A	V1B V2A
	2% Concentration (V2B)	V1A V2B	V1B V2B
	5% Concentration (V2C)	V1A V2C	V1B V2C

This study considered six (6) samples or solutions with variations in the essential oil concentration and the specific essential oil used. The factorial research design enabled the systematic and simultaneous investigation of how the two independent variables (essential oil used and concentration of essential oils) affect the research's single dependent variable (antibacterial activity). Thus, the design allowed the incorporation of the two independent variables to produce six antibacterial solutions that effectively manipulated these variables.

Furthermore, the factorial research design is suitable for the study as the research emphasized the most effective antibacterial solution from the experimentation. Therefore, their effects and interactions were properly measured, assessed, and evaluated to investigate their antibacterial properties. Additionally, the various subsections found in Table 1 allowed the researchers to observe every plausible mixture and note the effects of each sample for the given study.

Research Locale and Samples

Given its experimental nature, this study did not utilize a sample and sampling technique. Hence, six samples of antibacterial solution were considered, each composed of different possible mixtures of the ingredients. Additionally, the research took place in two locations: the residence of one of the researchers and the DOST-ITDI Microbiology Section Laboratory.

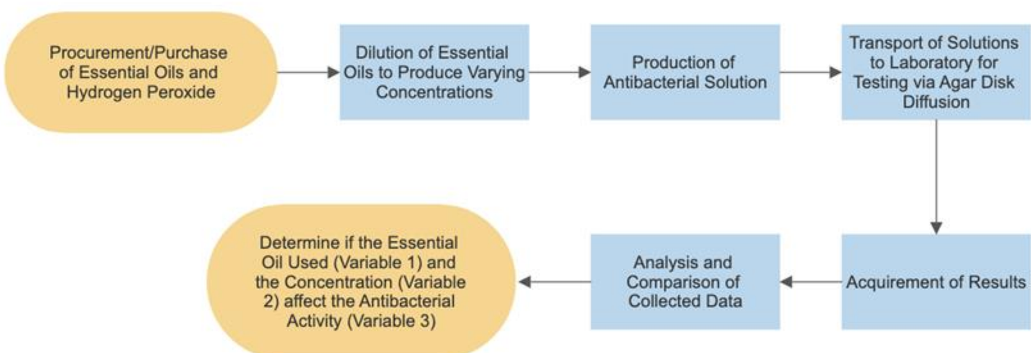
The recent outbreak of COVID-19 has restricted face-to-face interactions; hence, the purchase of materials and the formulation of the antibacterial solutions occurred in the assigned researcher's residence. The procedure was conducted on a clean, flat table in a room with air conditioning, with the use of gloves and masks as well. These precautions were essential to ensure efficiency in the work, limit inaccurate results, and comply with the proper safety and health regulations (Ausbright Facilities Management Melbourne, 2017). This process lasted a day at most.

The second location of this study was the DOST-ITDI Microbiology Section Laboratory. The Philippine government runs this laboratory and specifically tackles the microbiological testing of products like antibacterial solutions. Due to the lack of needed equipment and the threat of the coronavirus, the researchers were prevented from conducting the study personally in a laboratory; thus, the solutions created from Location 1 were delivered to the partner laboratory instead. Additionally, all communications with the laboratory were conducted online via emails or phone calls, and an adult leader was the one to deliver the samples to the laboratory. The results were then emailed to the researchers two to three weeks after the submission of the samples.

Data Gathering Procedures

Figure 2

Data-Gathering Flowchart



This study used of three main materials: hydrogen peroxide, cinnamon essential oil, and lemongrass essential oil. These materials were purchased through local stores by one of the researchers since the researchers could not manually extract the essential oils due to the restrictions of the pandemic. However, it was ensured that the essential oils purchased were manually extracted by manufacturers through steam distillation to ensure maximum quality.

To dilute the essential oils with sterile water, 10mL of water to 2, 4, and 10 drops of essential oil were used to produce concentrations of 1%, 2%, and 5%, respectively (NOW Foods, 2019). The six samples of antibacterial solution were produced by mixing 40mL of 3% hydrogen peroxide with the following: (1) a 1% concentration of cinnamon essential oil, (2) a 2% concentration of cinnamon essential oil, (3) a 5% concentration of cinnamon essential oil, (4) a 1% concentration of lemongrass essential oil, (5) a 2% concentration of lemongrass essential oil, and, lastly, (6) a 5% concentration of lemongrass essential oil. Thus, each sample of antibacterial solution contained 50mL. The researchers also created a control group of 50mL hydrogen peroxide for comparison. This control group contained no essential oils.

Figure 3

Antibacterial Solutions

Tightly sealed amber bottles with labels were then used to store these antibacterial solutions, and the bottles were sterilized with boiling water before their use. The amber bottles prevent oxidation and protect the essential oils from direct sunlight and heat. Afterward, the solutions were placed in a case or box for transport to the laboratory.

Figure 4

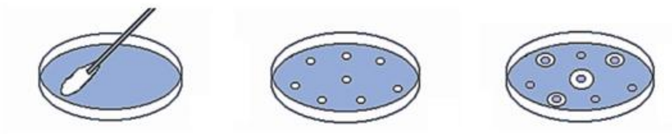
Sterilization of Amber Bottles

The solutions were transported to the DOST-ITDI Microbiology Laboratory, which performed the agar disk diffusion method to determine the antibacterial activity of the formulated samples. The laboratory personnel used large agar plates measuring approximately 150 mm, and each sample was tested against each of the two bacteria six times. The laboratory personnel also included a positive control (amikacin) and a negative control (sample-free disc) to regulate unknown variables and verify that no external factors affected the results. This further proved the integrity of the data derived from the research.

The bacteria were spread over the agar plates, and the antibacterial solutions were applied to the bacteria on each plate. The plates were then incubated at 37°C for 24 hours. Subsequently, the inhibition zones were measured millimeters using a ruler or caliper. The diameter of the inhibition zone is used to determine the efficacy of the solution's antibacterial activity against the bacteria used for testing. Recorded data from the laboratory and documentation of the experiment were sent to the researchers after two to three weeks through email.

Figure 5

Agar Disk Diffusion Method



Note. From Rao, S. (2006, June). Antibiotic susceptibility testing. *Department of Microbiology JJMMC.* https://www.microrao.com/micronotes/antibiotic.pdf

Data Analysis Procedures

The essential oil used, and the concentration of the essential oils are the study's independent variables. These were manipulated to investigate the antibacterial activity of the solutions. After the results were received from the laboratory, separate statistical treatments were performed to derive relevant conclusions to achieve the objectives presented at the beginning of the study. The data were analyzed through IBM SPSS Software version 26.

The data's mean and standard deviation were computed to determine the effect of the essential oils on the antibacterial activity of the disinfectant spray formulations despite varying concentrations of cinnamon or lemongrass essential oil. Mean pertains to the average or the most common value in a collection of numbers, whereas standard deviation measures how dispersed the data to the mean (Lee et al., 2015).

On the other hand, Multivariate Analysis of Variance (MANOVA) was employed to analyze which of the three concentrations of essential oils had a statistically significant effect on the antibacterial activity of the solutions. This statistical treatment is effective for analyzing multivariable models. Furthermore, it is often utilized for examining data with more than one independent variable and determining how quantitative variables can be combined to maximally compare distinct groups (Grice & Iwasaki, 2009).

The researchers used MANOVA to identify the effectiveness of the antibacterial solution with different essential oil concentrations. The application of this statistical treatment enabled the researchers to focus solely on the effects of the solutions on the bacteria used for testing. Hence, the positive control was removed to emphasize the antibacterial solutions. The negative control was also not included in the computations since its sole purpose was to verify that no external factors affected the results gathered.

RESULTS AND DISCUSSION

This research aims to formulate a safer and more effective disinfectant spray by investigating the effects of cinnamon and lemongrass essential oil on the antibacterial activity of hydrogen peroxide. The data gathered from the experimentation was subsequently analyzed to draw relevant conclusions in response to the research questions. The mean and standard deviation of the data, as seen in *Table 2*, was utilized to determine which of the two essential oils (cinnamon or lemongrass) had a more prominent effect on the antibacterial activity of the solutions. Meanwhile, the MANOVA technique was utilized to determine which of the three concentrations of essential oils inhibited the bacteria most effectively, as seen in Tables 3, 4, and 5.

Effects of Essential Oil Used on the Antibacterial Activity of the Disinfectant

Table 2

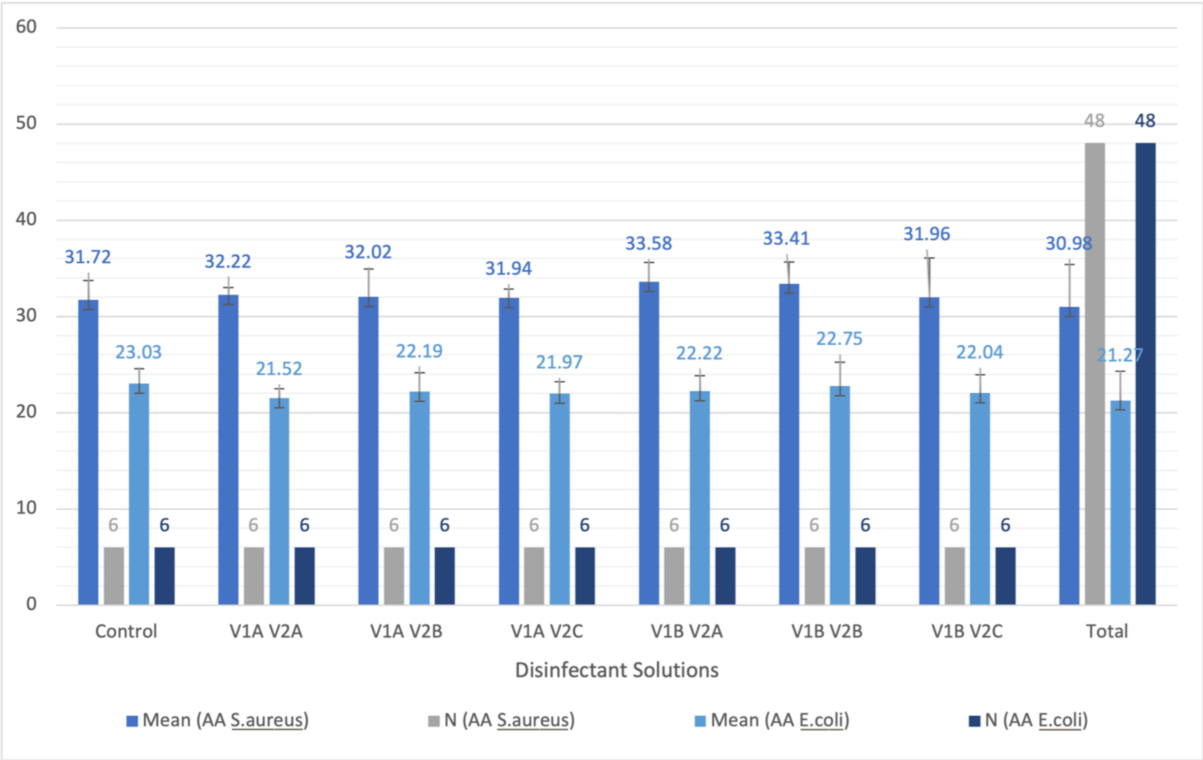
Mean and Standard Deviation of Antibacterial Solutions

	Disinfectant	Mean	SD	N		Disinfectant	Mean	SD	N
AA *S.aureus*	Control[a]	31.72	1.985	6	AA *E.coli*	Control[a]	23.03	1.532	6
	V1A V2A	32.22	.766	6		V1A V2A	21.52	.982	6
	V1A V2B	32.02	2.906	6		V1A V2B	22.19	1.982	6
	V1A V2C	31.94	.924	6		V1A V2C	21.97	1.240	6
	V1B V2A	33.58	2.006	6		V1B V2A	22.22	1.627	6
	V1B V2B	33.41	2.270	6		V1B V2B	22.75	2.481	6
	V1B V2C	31.96	4.114	6		V1B V2C	22.04	1.916	6
	Total	30.98	4.395	48		Total	21.27	3.047	48

Note. V1A V2A: 1% Cinnamon Essential Oil; V1A V2B: 2% Cinnamon Essential Oil; V1A V2C: 5% Cinnamon Essential Oil; V1B V2A: 1% Lemongrass Essential Oil; V1B V2B: 2% Lemongrass Essential Oil; V1B V2C: 5% Lemongrass Essential Oil; Control: No Essential Oils

Figure 6

Mean and Standard Deviation of Antibacterial Solutions



Note. V1A V2A: 1% Cinnamon Essential Oil; V1A V2B: 2% Cinnamon Essential Oil; V1A V2C: 5% Cinnamon Essential Oil; V1B V2A: 1% Lemongrass Essential Oil; V1B V2B: 2% Lemongrass Essential Oil; V1B V2C: 5% Lemongrass Essential Oil; Control: No Essential Oils

Table 2 displays the reactivity of the antibacterial solutions with either cinnamon or lemongrass essential oil against the bacteria used for testing. The left side of Table 2 corresponds to the antibacterial activity of all solutions against *Staphylococcus aureus*, where it is evident that the lemongrass samples (V1B) are more reactive to the bacteria than the cinnamon samples (V1A). From the lemongrass samples, the sample with the highest reactivity is V1B V2A (M = 33.58, SD = 2.006), followed by V1B V2B (M = 33.41, SD = 2.270) and V1B V2C (M = 31.96, SD = 4.114).

Among the samples with cinnamon essential oil, the one with the highest antibacterial activity is V1A V2A (M = 32.22, SD = 0.766), followed by V1A V2B (M = 32.02, SD = 2.906) and V1A V2C (M = 31.94, SD = 2.906).

Consequently, the right columns of Table 2 demonstrate the reactivity of all antibacterial solutions against *Escherichia coli*. Based on the mean of each solution, the sample with the highest antibacterial activity is V1B V2B (M = 22.75, SD = 2.481), followed by V1B V2A (M = 22.22, SD = 1.627) and V1A V2B (M = 22.19, SD = 1.982). Following these are V1B V2C (M = 22.04, SD = 1.916), V1A V2C (M = 21.97, SD = 1.240), and V1A V2A (M = 21.52, SD = 0.982). As such, it can be noted that the lemongrass samples still have higher antibacterial activity than the cinnamon samples, except that sample V1A V2B was more effective than V1B V2C against *Escherichia coli*.

Based on the data, it is evident that the essential oil used affected the antibacterial activity of the disinfectant spray formulation, though by only a relatively small margin compared to the control group that did not contain any concentration of essential oils. The control group provides a reliable reference for how the essential oils improve the solutions' antibacterial activity. Of the two, the lemongrass essential oil was more effective at increasing antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli* bacteria. It is followed by cinnamon essential oil closely, with their disparity being only a minimal amount.

It can also be noted that, though not included in the data table, the mean for the negative control is 0. With the negative control being a sample-free disc, this data validates that no external factors affected the experiment's outcome. The data gathered is reliable and free from any biases or errors that could have occurred during the data-gathering procedures.

Effects of Varying Concentrations of Essential Oils on the Antibacterial Activity of the Disinfectant

Table 3

Box's Test of Equality of Covariance Matrices and Test of Normality

Box's Test of Equality of Covariance Matrices					Shapiro-Wilk			
Box's M	F	df1	df 2	Sig.	Standardized residual	Statistic	df	Sig
24.445	1.129	18	5306.150	.315	Antibacterial activity against S.aureus	.989	42	.957
					Antibacterial activity against E.Coli	.972	42	.390

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Disinfectant

The Box's Test in Table 3 shows that the assumption on the equality of variance is satisfied, $p > 0.05$. The normality of the standardized residuals was examined using Shapiro-Wilk Test, and it found that the residuals follow a multivariate normal distribution ($p > 0.05$). Hence, Wilk's Lambda will be used to determine if there is a significant difference in the antibacterial activity of the six solutions of 3% hydrogen peroxide with cinnamon or lemongrass essential oil at concentrations of either 1%, 2%, or 5% against *S.aureus* and *E.coli*.

Table 4

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared[d]
Disinfectant	Pillai's Trace	.173	.552	12	70	.872	.086
	Wilks' Lambda	.835	.536	12	68	.883	.086

a. Design: Intercept + Disinfectant

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Computed using alpha = .05

d. Partial eta squared can be cited as a measure of effect size: f^2 is Cohen's effect size: .02 = small, .15 = moderate, .35 = large.

Furthermore, Table 4 presents the results of the difference between the antibacterial activity of the disinfectants against *S.aureus* and *E.coli*. This study found no statistically significant difference in the antibacterial activity of the six solutions with either cinnamon or lemongrass essential oil at concentrations of 1%, 2%, or 5% against the two bacteria (*S.aureus and E.coli*), *F (12, 70) = 0.536, p > 0.05, Wilks' Lambda = 0.835, partial = 0.086.*

Table 5

Test Between-Subjects Effects

	Antibacterial activity against	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared[a]
Disinfectant	*S.aureus*	20.797	6	3.466	.608	.722	.094
	E.Coli	9.110	6	1.518	.500	.804	.079

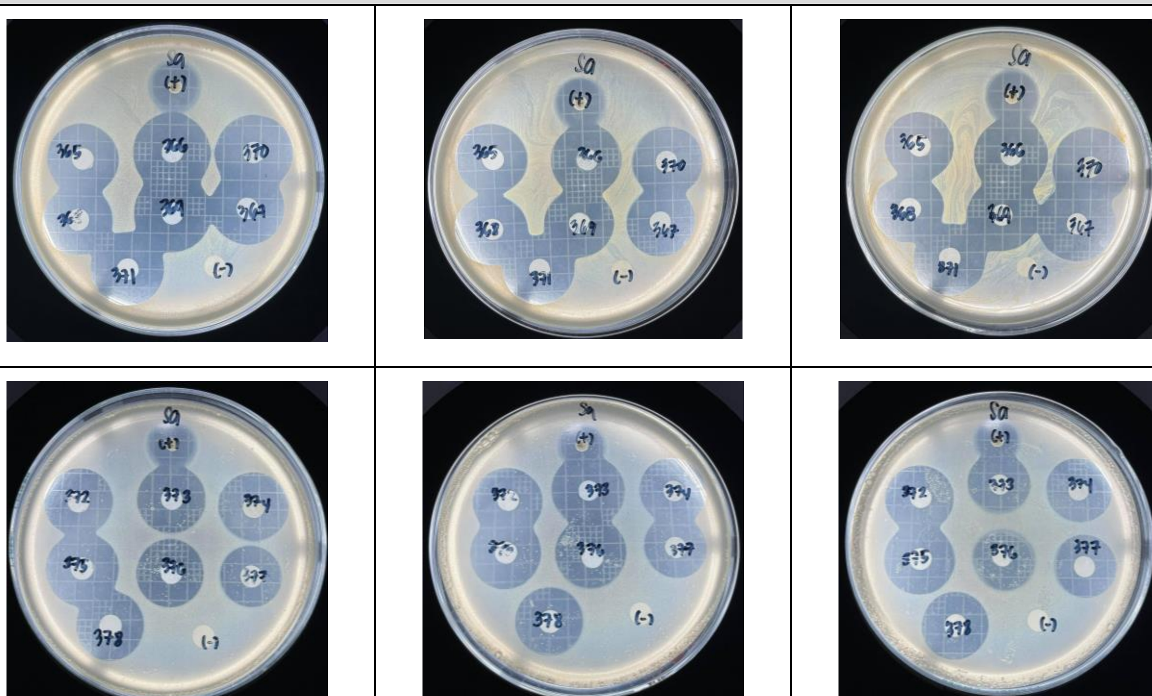
a. Partial eta squared can be cited as a measure of effect size: is Cohen's effect size: .02 = small, .15 = moderate, .35 = large.

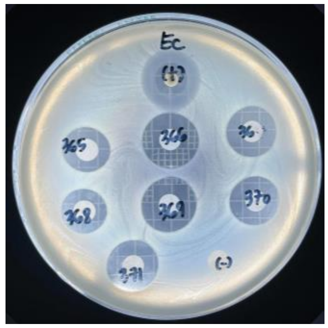
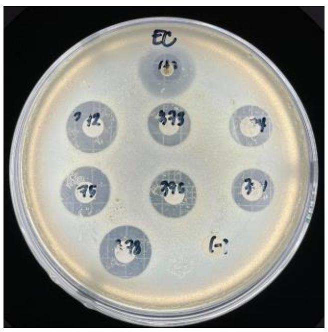
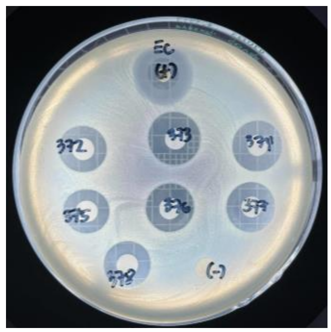
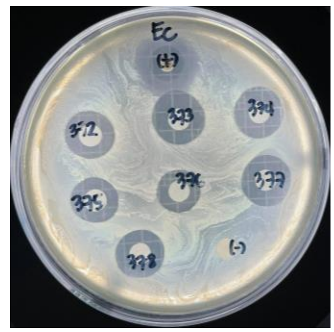
Consequently, Table 5 shows that the antibacterial activity of the disinfectants had a small effect on both *S.aureus* (*partial* = 0.094) and *E.coli* (*partial* = 0.079). However, this effect was not statistically significant on the antibacterial activity against *S.aureus* and *E.coli* *($p > 0.05$)*.

Figure 7

Agar Disk Diffusion Performed on the Antibacterial Solutions (Images taken by the DOST-ITDI Microbiology Laboratory)

Staphylococcus aureus



Escherichia coli		
		
		

Additionally, in Figure 7 are 12 images that display the results from the agar disk diffusion method performed by the DOST-ITDI Microbiology Laboratory.

The 12 images can be categorized into two. Six images exhibit how the antibacterial solutions reacted to *Staphylococcus aureus*, while the other six images depict their reactions to *Escherichia coli*. In addition, one agar plate is equivalent to one replicate or trial of all the antibacterial solutions, resulting in six replicates or trials under each bacteria. From left to right, top to bottom, both categories show sample replicates 1 to 6.

Moreover, the following are the labeled samples together with their corresponding sample code in the documentation:

V1A V2A: 368, 375

V1A V2B: 369, 376

V1A V2C: 365, 372

V1B V2A: 367, 374

V1B V2B: 366, 373

V1B V2C: 370, 377

Control: 371, 378

Positive Control: (+)

Negative Control: (-)

Summary of Results

In summary, Table 2 reveals that lemongrass essential oil is more effective than cinnamon essential oil at increasing the solutions' antibacterial activity based on the samples' mean and standard deviation. Moreover, Table 4 determined that there was no significant difference between the antibacterial activity of the disinfectants against *S.aureus* and *E.coli* bacteria, whereas Table 5 pointed out that the antibacterial activity of the disinfectants only had a small and statistically insignificant effect on the chosen bacteria (*S.aureus* and *E.coli*).

Discussion

From the statistical treatments performed on the gathered data, it is evident that adding lemongrass or cinnamon essential oil at concentrations of either 1%, 2%, or 5% improved the antibacterial activity of the solutions by a comparatively minimal margin against *Staphylococcus aureus* and *Escherichia coli* bacteria. This is with reference to the control group that incorporated no concentration of cinnamon or lemongrass essential oil.

Effects of Essential Oil Used on the Antibacterial Activity of the Disinfectant

This study sought to answer how the essential oil used (cinnamon or lemongrass) can affect the antibacterial activity of the disinfectant spray formulation. Based on the data gathered, it was found that the essential oil used had a marginal impact on the antibacterial activity compared to the control group that did not contain any concentration of the essential oils, which served as a reference for this study. With a minimal gap, it was discovered that the lemongrass samples were effective at increasing antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* compared to the cinnamon samples, except sample V1A V2B had a higher reactivity compared to V1B V2C against *E. coli* bacteria. Nonetheless, the gathered data aligned with the conceptual framework in that adding essential oils increased the antibacterial activity of the hydrogen peroxide solutions. These findings are consistent with the findings of Raeisi et al. (2015), Shabani et al. (2016), and Jafari et al. (2012).

Raeisi et al. (2015) performed a research study that evaluated the antimicrobial effect of cinnamon essential oil, monolaurin, nisin, and ethylenediaminetetraacetic acid on *Staphylococcus aureus* and *Escherichia coli.* The results from the GS/MS analysis showed the presence of a high concentration of cinnamaldehyde, which is believed to be a major component of the cinnamon essential oil. The information gathered from the previous study, similar to the current data, adequately portrayed the potential of cinnamon essential oil in increasing the antibacterial activity of disinfectant spray formulations.

Another study by Shabani et al. (2016) investigated the antimicrobial activity of cinnamon oil against *Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* in three different concentrations (25%, 50%, 100%) through the disk diffusion method. According to the results, cinnamon oil was a better antimicrobial agent, given its broad range of antimicrobial activity against *S.aureus*, *S.pyogenes*, and *S.epidermidis,* than common chemical antibiotics. Therefore, the findings from this study strengthened the credibility of the information and data gathered.

In a research conducted by Jafari et al. (2012), it was stated that lemongrass was known to be a medicinal plant in the past that was used in the treatment of various diseases, such as neurological and gastrointestinal disorders. It was stated that lemongrass essential oil has the potential to control bacterial growth and limit fungal pollutants such as *Staphylococcus aureus* and *Escherichia coli*. The study by Jafari et al. (2012) attests to the strong antibacterial activity of lemongrass essential oil observed in this study, especially since lemongrass samples were more effective at inhibiting bacteria than the cinnamon samples.

Consequently, a study by Zulfa et al. (2016) focused on the antimicrobial activity of methanolic *Cymbopogon citratus* (lemongrass). Extracts were tested against five foodborne pathogens, including *Escherichia coli* and *Staphylococcus aureus*. Based on their observations, the inhibition zones produced by the lemongrass measured 7.5 mm and 10 mm against *E.coli* and *S.aureus*, respectively. This study had a similar methodology to this one, and it is evident that the lemongrass solution has a high antibacterial activity against the bacteria used for testing. It can also be observed that the essential oils were more effective and had larger inhibition zones against *S.aureus* than *E.coli*. This is true for both this study and the study by Zulfa et al. (2016), indicating that gram-positive bacteria like *S.aureus* are more reactive to essential oils than to gram-negative bacteria like *E.coli*. This pattern was observed in similar studies previously cited.

Lastly, a study by Singh et al. (2011) investigated the antimicrobial effects of lemongrass essential oil against *Escherichia coli* and *Staphylococcus aureus*. Through the disc diffusion method, it was found that 38.2% of the 1,114 strains were reactive to the said essential oil.

Consequently, this data supported the high levels of antibacterial activity exhibited by the lemongrass essential oil against *E.coli* and *S.aureus* bacteria.

Both cinnamon and lemongrass essential oils have shown antibacterial activity against *E.coli* and *S.aureus* bacteria, according to the related literature. Though it was found that the lemongrass samples generally had a higher antibacterial activity compared to the cinnamon samples*,* the slight disparity between the two essential oils may be due to the phenols and alkaloids found in lemongrass essential oil (Jafari et al., 2012). However, the statistically insignificant difference between the inhibition zones of the lemongrass and cinnamon samples implies that the inclusion of these essential oils made a minimal difference in the antibacterial activity of the solutions. For future manufacturers intending to formulate solutions with these essential oils, either can be utilized, given their negligible disparity in antibacterial properties. Despite this, future researchers are encouraged to pursue other potentially effective materials in reducing pathogens.

Effects of Varying Concentrations of Essential Oils on the Antibacterial Activity of the Disinfectant

This study sought to determine how varying concentrations of the essential oils used would influence the antibacterial activity of the disinfectant spray formulation. Based on the data gathered through disk diffusion, it was found that there was no statistically significant difference in the antibacterial activities of the six solutions with different concentrations of lemongrass or cinnamon essential oil against *S. aureus* and *E.coli*. Among the measurements of inhibition zones, a concentration of 1% essential oil was most potent against *S.aureus* bacteria, whereas the concentration of 2% essential oil was most effective against *E.coli* bacteria. These findings were unexpected, as they contradicted the initial hypothesis that a greater concentration would result in greater antibacterial activity. However, this neither invalidated the research nor negated its significance to the field of knowledge.

According to Cattelan et al. (2013), 1%, 2%, and 5% oregano essential oil concentrations were tested for their antibacterial activity against six different bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The researchers used disc diffusion and well-diffusion methods, and the results found only minimal differences in the antibacterial activities of the three concentrations used. However, the 5% concentration was the most potent by a small margin. Though the latter result is different, the difference in which concentration is most effective may be due to the different essential oil used. The varying result may also be due to the difference in the quality of the lemongrass and cinnamon essential oils used, given that they were purchased and not manually extracted due to the Covid-19 pandemic.

Furthermore, Valeriano et al. (2012) likewise utilized peppermint and lemongrass essential oil as ingredients of a disinfectant. Concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.8, and 3.9 $\mu L/mL^{1}$ were used. The disinfectants were tested by applying the bacteria on a stainless steel surface, and then applying the disinfectant on the surface. The study found that disinfectants mixed with essential oils could reduce the microbial colonies on the surface with the 500 $\mu L/mL^{1}$ concentration of lemongrass essential oil being the most effective. It again varies with the findings of this study, as the greatest concentration of lemongrass essential oil was the most effective concentration in the study by Valeriano et al. (2012).

Zainol et al. (2017) conducted a study where the researchers used cinnamon and clove essential oil diluted in dimethylsulfoxide at the following concentrations: 1.25 mg/mL, 0.62 mg/mL, 0.31 mg/mL, 0.15 mg/mL, 0.078 mg/mL, 0.039 mg/mL, 0.019 mg/mL, and 0.00 mg/mL. The research sought to test the synergistic effects of the two essential oils against resistant oral pathogenic bacteria. The solutions were then added to a 96-microtiter plate containing the bacteria. The study found that the solutions only showed partial synergy when tested on *S.salivarius*, *E. faecalis*, and *S.mutans*.Similar to the current study, adding the essential oils at different concentrations did not greatly affect the solution's antimicrobial activity.

In a research conducted by Mathew et al. (2016), the researchers used diluted lemongrass essential oil mixed with sterile water in concentrations of 1:1, 1:2, and 1:4. The surface disinfection test was performed. The results found that the diluted lemongrass essential oil was just as effective as the control group, which was the disinfectant Lysol. Compared to the current study, Mathew et al. (2016) involved diluted lemongrass essential oil that was not mixed with hydrogen peroxide, yet its antibacterial properties were already on par with that of the control group. Thus, perhaps another reason for the lack of significant difference is the minimal synergy between essential oils and hydrogen peroxide.

According to research conducted by Puškárová et al. (2017), the researchers used six essential oils: oregano, thyme, clove, lavender, clary sage, and arborvitae. These essential oils The Mueller-Hinton disk diffusion method found that the essential oils were very effective against all of the bacterial strains involved in the experiment, with inhibition zones ranging from 26–54 mm. The results of the study by Puškárová et al. (2017) likely differ from this study due to the different concentrations of essential oil used and the different substances used to dilute the essential oils.

Nonetheless, the lack of any statistically significant difference in the antibacterial activity of the different concentrations implied that adding essential oils would not heavily improve the effectiveness of an antibacterial solution. This finding is consistent with the findings of Cattelan et al. (2013) and Zainol et al. (2017), which can indicate that these properties are not fully maximized or utilized as components of a disinfectant spray. This can encourage future researchers to investigate other components that can likely increase the antibacterial activity. Consequently, it can discourage disinfectant manufacturers from making costly efforts to include essential oils if they intend to produce an effective disinfectant since it will have no notable effect.

CONCLUSIONS

This research endeavored to formulate an effective and more affordable disinfectant spray using hydrogen peroxide, lemongrass essential oil, and cinnamon essential oil. It was initially hypothesized that including essential oils and using higher concentrations would increase the antibacterial activity of the solution extensively. However, the results revealed that the inclusion of the essential oils had no statistically significant effect on the antibacterial activity of the hydrogen peroxide solution, regardless of the concentration used. It was also found that the lemongrass samples were more effective at inhibiting bacteria than the cinnamon solutions, though only by a small margin.

Though the effects of the essential oils on the antibacterial activity of the solutions were found to be insignificant, it can be noted from this research that the inclusion of essential oils should be minimally considered when creating a maximally effective disinfectant spray, since the difference in antibacterial activity made by this component is relatively inappreciable. Moreover, manufacturers may also neglect to add such essential oils, as the aforementioned substance does not significantly change effectiveness against bacteria. It can also be deduced that hydrogen peroxide and essential oils have low synergy, although hydrogen peroxide already possesses extensive antibacterial properties.

Furthermore, the objectivity of the research is in line with the limitations presented, specifically as influenced by the factors that have resulted in unexpected findings and variables left to chance, such as the difference in the quality of the essential oils purchased, considering that the researchers themselves did not manually extract the materials. Errors in agar disk diffusion by the laboratory are unlikely because of the negative control (sample-free disc). As a future direction for research, researchers may investigate other substances that can potentially increase the antibacterial activity of a disinfectant spray solution or find other components that have more synergy with the materials used in this research. Should essential oils be used in future research, it is recommended to extract these to ensure maximum quality manually. Lastly, future researchers may also use various antibacterial tests against a wide range of bacterial populations to further affirm the antibacterial solution's capacity to inhibit pathogens.

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