ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

# In Vitro Assessment on the Synergistic Antibacterial Effect of Ginger (*Zingiber officinale* Roscoe) Extract with Clindamycin Antibiotic against *Staphylococcus aureus*

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Publication Date: 2025/04/14

**Abstract:**

- ***Objectives:***

**Examine if there is a synergistic antibacterial effect of Ginger (*Zingiber officinale* Roscoe) extract with clindamycin antibiotic against *S. aureus*.**

- ***Methods:***

**The ginger extract was prepared by air-drying 50 grams of ginger and macerating it in 500 mL of 95% ethanol for 24 hours, followed by filtration and evaporation to obtain a condensed extract. Staphylococcus aureus colonies were inoculated on nutrient agar and incubated at 37°C for 18 to 24 hours. A 1 mg/mL clindamycin suspension was prepared from 300 mg capsules. MHA plates were inoculated with S. aureus, and filter paper discs labeled Clindamycin (C), Ginger (G), and combinations (GC50, GC75, GC100) were placed on the agar. The plates were incubated at 37°C for 18 to 24 hours, and the zones of inhibition were measured using the Kirby-Bauer Test to assess the synergy through mean ZOI and cooperative effect synergy testing.**

- ***Results:***

**The results of the study indicate that the combination of ginger extract and Clindamycin generally showed antagonistic interactions. However, GC100 (100% ginger extract and Clindamycin) had a mean zone of inhibition (ZOI) closest to Clindamycin alone, suggesting potential antibacterial benefits at higher concentrations of ginger extract. Further analysis using cooperative effect synergy models confirmed the lack of synergistic interaction. Pairwise comparisons revealed that GC50 (50% ginger extract and Clindamycin) had a significant difference in effectiveness compared to Clindamycin alone (p-value: 0.045), while GC75 (75% ginger extract and Clindamycin) and GC100 did not exhibit significant differences.**

- ***Conclusion:***

**These findings underscore the complexity of combining natural extracts with antibiotics. Although the combination did not enhance antibacterial efficacy, the potential of high-concentration ginger extract to act as a complementary treatment warrants further investigation to develop effective strategies against antibiotic-resistant *S. aureus*.**

***Keywords:*** *Antibacterial Effect, Clindamycin, Ginger, Synergy, S. aureus*.

**How to Cite:** Lady Jane A. Bacnotan; Mark Albert A. Gumapon; Christine Joy M. Inotao; Shaina Lyn T. Yarot; Junge B. Guillena; Cath Joy B. Ronquillo, RMT (2025). In Vitro Assessment on the Synergistic Antibacterial Effect of Ginger (*Zingiber officinale* Roscoe) Extract with Clindamycin Antibiotic against *Staphylococcus aureus*. *International Journal of Innovative Science and Research Technology*, 10(3), 2762-2772. https://doi.org/10.38124/ijisrt/25mar816

## I. INTRODUCTION

On a global scale, Staphylococcus aureus (*S. aureus*) stands as a prominent contributor to infections acquired both in healthcare settings and within communities (Goudarzi et al., 2020). *S. aureus* is an exceptionally common pathogenic microorganism in humans, capable of instigating a range of infectious ailments including skin and soft tissue infections, endocarditis, osteomyelitis, bacteremia, and life-threatening pneumonia. Under normal circumstances, S. aureus does not

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

usually lead to infections on healthy skin. Nevertheless, if it gains access to the bloodstream or internal tissues, these bacteria have the potential to trigger a range of potentially severe infections (Lowy, 1998).

In the study of Ikuta et al. (2022), 7.7 million deaths worldwide were attributed to bacterial pathogens. *S. aureus* was identified as one of the top five causative agents of the 7.7 million deaths worldwide in 2019. S. aureus emerged as the primary bacterial cause of death in 135 countries and was linked to the highest number of deaths in individuals aged 15 years and above on a global scale.

According to the Centers for Disease Control and Prevention (CDC) in 2019, over 119,000 individuals experienced bloodstream *S. aureus* infections, and nearly 20,000 of them lost their lives in the United States in 2017. In the Philippines, MRSA infection rates have consistently exceeded 50% since 2010 (Masim et al., 2021). In the study of Valle et al. (2016), 236 S. aureus bacteria were obtained from clinical samples at Makati Medical Center in Makati City, Philippines, during the period from January 2013 to June 2013 and 108 of them were identified as MRSA. In Iligan City, the study of Maratas and Cuadra (2017) discovered that 73 out of the 114 children who participated were carrying *S. aureus* in their nasal passages indicating the presence of the bacteria in the local setting.

Antimicrobial drugs are essential in lowering the burden of infectious diseases worldwide. However, the widespread utilization of antibiotics has heightened the selective influence on bacteria, leading to the development of drug resistance. This antibiotic resistance in bacteria represents a significant worldwide risk to public health (Coates et al., 2014). The transmission and expansion of these resistant strains diminish the effectiveness of antimicrobial treatments, thereby extending hospitalization periods, escalating treatment expenses, and raising the mortality rate.

*S. aureus* mutated due to bacterial evolution and excessive antibiotic use, resulting in global prevalence of MRSA infections that has increased because of increasing antibiotic resistance, which presents significant hurdles for clinical anti-infective treatment (Guo et al., 2020). This bacterial resistance to antibiotics poses a serious threat to public health, and rates of resistance are rising globally, especially for critical last-resort antibiotics (Levy & Marshall, 2004). The resistance to methicillin is a frequently encountered mechanism in *S. aureus*. Shortly following the initial clinical application of methicillin, the swift emergence of methicillin-resistant S. aureus (MRSA) has restricted the available therapeutic options for treating MRSA infections (Appelbaum, 2007).

Antibiotics such as vancomycin, linezolid, quinupristin, and dalfopristin have traditionally been the preferred choice for the management of S. aureus. However, the growing instances of resistance to these antibiotics have heightened concerns about their effectiveness (Cong et al., 2020). Due to heightened doubt about the effectiveness of traditional antibiotics, clinicians are increasingly turning to the macrolide lincosamide-streptogramin B (MLSB) family as an alternative for treating S. aureus infections. Among the MLSB family, clindamycin is an ideal antibiotic due to its favorable pharmacokinetics (Gemmell et al., 2006).

Clindamycin is a lincosamide antibiotic used to treat serious infections caused by susceptible anaerobic, streptococcal, staphylococcal, and pneumococcal bacteria. One of its notable attributes is its ability to suppress the production of toxins in strains of streptococci and staphylococci that produce toxins. Clindamycin also accumulates in high levels within cells that engulf pathogens (phagocytic cells) and in bone tissue, making it of particular interest for certain applications. Due to its impressive pharmacokinetic characteristics and effectiveness against MRSA, clindamycin is the preferred choice for treatment, according to Klempner and Styrt (1981). Choosing clindamycin from the MLSB family is preferable due to its lower cost, reduced side effects, and superior tissue penetration. The availability of generic forms of clindamycin can make it more affordable for patients and healthcare facilities (Valle et al., 2016). With the rise of antibiotic resistance and due to excessive usage, S. aureus becomes increasingly resistant to clindamycin (Juayang et al., 2014).

In the Philippines, cultural misconceptions and inadequate regulation and enforcement of antibiotic use have shaped provider and patient attitudes and behavior. Self-medication is rampant in the Philippines, with a prevalence of 31–66% which is a major contributor to the development of antibiotic resistance (Thapa et al., 2021).

According to Juayang et al. (2014), clindamycin serves as a viable option for treating MRSA and methicillin-sensitive Staphylococcus aureus (MSSA) infections. However, the results of the study revealed a substantial inducible resistance to clindamycin in S. aureus, reaching as high as 56.4%. Out of 94 cases diagnosed with S. aureus infections, 37 were inducible clindamycin resistant at a tertiary hospital in the Philippines. Despite clindamycin being an effective antibiotic for staphylococcal infections, its high resistance rate poses a challenge, making it less reliable for ensuring successful treatment.

With the rise of antibiotic resistance including clindamycin, the development of alternative antibacterial strategies is critical. It is crucial to urgently create new antibiotics that can combat bacteria and prevent them from developing resistance (Haroun & Al-Kayali, 2016). This is necessary to improve the effectiveness of treatments against these resistant strains. This circumstance has made people reevaluate the therapeutic potential of traditional cures like plants and plant-based products (Mandal et al., 2010).

Plants have served as a valuable source of natural substances for promoting human health for a significant span of time. According to the World Health Organization (2013), medicinal plants are considered an excellent source for obtaining a wide range of drugs. Plant extracts hold significant promise as antimicrobial agents against various microorganisms, offering potential solutions for treating

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

infectious diseases caused by drug-resistant microbes. Plant antimicrobials, while not inherently antimicrobial, can enhance the effectiveness of standard drugs when used together. This synergistic effect underscores the potential benefits of combining these plant compounds with conventional medications for improved outcomes (Hübsch, 2014). Combination therapy or synergistic therapy, employed against drug-resistant microorganisms, has the potential to open up new avenues for treating infectious diseases (Chanda & Rakholiya, 2011).

One of the most popular spice crops is ginger (Zingiber officinale), which is valued for its therapeutic properties. It is an herbaceous perennial plant of the Zingiberaceae family (Sharma, 2017). Throughout the world, ginger is a widely used medicinal plant for a wide range of illnesses (Ali et al., 2008). Ginger may act as an antimicrobial agent and hence could be used for treating a number of bacterial diseases (Tan & Vanitha, 2004). Ginger is relatively less expensive, readily available, widely accepted, and tolerated by the majority of people. Reported pharmacological activities on ginger involve antioxidant, anti-inflammatory, antimicrobial, antinociceptive and hepatoprotective (Abdel Azeem et al., 2013; Mostafa and Singab, 2016). Ginger is known for its strong antibacterial and fewer antifungal properties. The bioactive molecules present in ginger include phenolic acids, terpenes, and flavonoids (Ghasemzadeh et al., 2010) which contribute to the various health benefits associated with ginger, including its antibacterial properties. Zingiber officinale Roscoe is one of the most commonly used herbs in Asia, and has been empirically used to treat disorders (Syafitri et al., 2018).

This study aims to determine if there is a synergistic antibacterial effect of Ginger (Zingiber officinale Roscoe) extract with clindamycin antibiotic against S. aureus. In this study, the researchers' goal is to develop an alternative by combining clindamycin with ginger extract that produces a combinational effect that is greater than the sum of its individual elements. The primary focus of the study is the use of ginger extract through ethanolic extraction and the clindamycin antibiotic and determine its zone of inhibition which was compared to when clindamycin and ginger are used alone.

## II. MATERIALS AND METHODS

➢ *Ginger Preparation*

- *Air Drying of Ginger*

In this study, 1,000 grams of ginger (Zingiber officinale Roscoe) was purchased locally in Tambo Public Market in Iligan City. The ginger was cleaned with running water, cut into small pieces, and was air dried following the procedure of Arias et al. (2004).

- *Ethanolic Extraction of Sample using Rotary Evaporator*

50 grams of dried ginger was macerated into 500 mL of 95% ethanol in a large container for 24 hours with occasional stirring for the first 6 hours. The ratio of ginger in grams to ethanol in mL follows the 1:10 weight/volume as described in the study. After maceration, the sample was filtered using a filter paper to remove any excess residue. It was subsequently subjected to a rotary steam evaporator operating at a temperature of 40°C for 2 hours, resulting in the production of a condensed extract. The obtained yield was weighed and recorded

➢ *Test Organism (S. aureus) Collection and Preparation*

The test microorganism that was used in this study is S. aureus. The test organism was a clinical isolate and obtained from the laboratory in Adventist Medical Center-Iligan. Isolated S. aureus colonies were inoculated in nutrient agar (NA) and incubated with a temperature of 37°C for a period of 18 to 24 hours. Preparation of S. aureus colonies from the nutrient agar was done through the streaking-plate method.

- *Antibiotic Collection*

Clindamycin was purchased in the Eagle's Pharmahealth and was in a capsulated form, weighing 300 mg.

- *Antibiotic Suspension Preparation*

The concentration that was used for the antibiotic suspension was 1 mg per mL following the study of Maano (2023). Clindamycin HCL is a water-soluble drug according to Chaiwarit et al. (2020), so the 300 mg clindamycin capsule was diluted in a 300 mL sterile distilled water to yield 1 mg/mL of suspension.

- *Preparation of the Inoculum*

To create suspensions of the test organism, a sterile inoculating loop was used to gather four to five isolates of S. aureus, which was then suspended in a test tube containing sterile distilled water. The suspension was adjusted to the 0.5 McFarland standard using a Wickerham card for comparison.

- *Inoculation of Bacterial Suspension in MHA using Lawn Method*

Once the suspension for the test organisms were prepared, they were inoculated onto the prepped Mueller-Hinton agar plates using a sterile swab, with two streaks over the agar surface. 9 MHA plates were utilized in the study for susceptibility testing of 3 replicates each of different concentrations of antibiotic-extract combination, 3 replicates of ginger, and 3 replicates of clindamycin as control variables.

- *Placement of Antibiotic Discs and Dispensing of Antibiotic-Extract Combination*

Small paper discs measuring 6mm in diameter on MHA were created in Whatman number one (No. 1) filter paper. These discs have undergone sterilization to eliminate any potential contaminants that could affect the outcomes. Employing forceps, the discs were placed individually onto the MHA agar surface, each with its corresponding markers at the plate's bottom for simple recognition. Discs were labeled as clindamycin (C), Ginger (G), 50% Ginger and clindamycin combination (GC50), 75% Ginger and clindamycin combination (GC75), 100% Ginger and clindamycin combination (GC100). After placing the discs

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

onto all the plates, clindamycin and various concentrations of ginger extract were prepared as shown in Table 1.

When dispensing the antibiotics and extract onto the paper discs, a 10µL micropipette was utilized. Each disk received a total of 10µL of liquid. This means that in the case of a 100% antibiotic-extract combination, both the antibiotic and the extract was 5µL each. The same principle applies to the 50% and 75% extract concentrations, which was achieved by diluting the plant ethanolic extract with 10% DMSO following the study of Maano (2023).

Table 1 Amount of Concentrations of Ginger, DMSO, and Clindamycin in Antibiotic-Extract Combination

| | 50% Ginger Concentration | 75% Ginger Concentration | 100% Ginger Concentration |
|---|---|---|---|
| Ginger (µL) | 2.5µL | 3.75µL | 5µL |
| DMSO (µL) | 2.5µL | 1.25µL | 0µL |
| Clindamycin (µL) | 5µL | 5µL | 5µL |
| TOTAL | 10µL | 10µL | 10µL |

One MHA plate catered to the 1st replicate of 50%, 75%, and 100% ginger contractions. Another MHA plate was used each for the 2nd and 3rd replicates of 50%, 75%, and 100% ginger contractions. One MHA plate was used for the 1st replicate of clindamycin and another MHA plate was used for ginger alone as control variables. Another MHA agar plate was used each for the 2nd and 3rd replicates of clindamycin and ginger as control variables. A total of 9 MHA plates was utilized; 3 of which were used for 3 replicates of antibiotic-extract combination, another 3 were used for 3 replicates of ginger alone, and 3 plates were used for 3 replicates of clindamycin alone. Subsequently, the plates were incubated with a temperature of 37°C for a period of 18 to 24 hours. Its zone of inhibition was assessed.

➢ *Organoleptic Evaluation of the Ginger Extract*

Organoleptic evaluation refers to the sensory assessment of the ginger extract. The color, consistency, aroma profile, and its intensity were assessed through organoleptic analysis by the researchers.

➢ *Testing of Antibacterial Activity*

- *Zone of Inhibition Measurement using Kirby-Bauer Test*

The antibacterial effectiveness was assessed on Mueller-Hinton Agar (MHA) using the Kirby-Bauer disk diffusion susceptibility method, following the procedure outlined by Hudzicki (2009) as published in the American Society for Microbiology. Zone of Inhibition Test (also referred to as Kirby-Bauer test) is used to determine the susceptibility or resistance of pathogenic bacteria to antibacterial agents.

In the study, a ruler was used in measuring the diameter of the zone of inhibition of each disk. The zones of inhibition of each disk was recorded. The ZOIs of the 3 replicates of the same concentration were added and divided by the number of replicates to get the mean ZOI. This also applies to the 3 replicates of clindamycin and ginger in order to get the mean ZOI. The concentration with the highest mean ZOI signifies a higher antibacterial activity than the rest of the concentrations.

$$Mean\ ZOI = \frac{ZOI\ of\ Replicate\ 1 + ZOI\ of\ Replicate\ 2 + ZOI\ of\ Replicate\ 3}{No.\ of\ Replicates\ (3)}$$

- *Synergy Determination using Cooperative Effect Synergy*

To determine if there is synergism, indifference, or antagonism in the combination of extract and antibiotic, the Cooperative Effect Synergy as described by Geary (2013). ZOI(G) and ZOI(C) are the mean zone of inhibition of the control agents, Ginger and clindamycin, when applied individually. ZOI(GC50), ZOI(GC75), and ZOI(GC100) are the mean zone of inhibition of the antibiotic-extract combination with its respective concentrations. The quantitative definition is that Ginger and clindamycin synergize if ZOI(GC) > ZOI(G) and ZOI(GC) > ZOI(C).

The antibiotic-extract combination is synergistic if the zone of inhibition of the combination is greater than the zone of inhibition of the control agents. Additionally, the antibiotic-extract combination is antagonistic if the zone of inhibition of the combination is lesser than the zone of inhibition of the control agents. The antibiotic-extract combination with the highest zone of inhibition has the highest synergistic property.

Synergistic: ZOI(GC) > ZOI(G) and ZOI(GC) > ZOI(C)

Antagonistic: ZOI(GC) < ZOI(G) or ZOI(GC) < ZOI(C)

Additive: ZOI(GC) = ZOI(G) +ZOI(C)

➢ *Statistical Treatment of Data*

- *Shapiro-Wilk (Normality) Test*

The Shapiro-Wilk test assesses whether a dataset is normally distributed. The test statistic *W* measures the fit to a normal distribution, ranging from 0 to 1. A lower *W* suggests departure from normality. The p-value indicates the probability of obtaining *W* under the null hypothesis of normality. If $p >$ significance level, the data may be normally distributed. Deviation from normality may require non-parametric tests or data transformation for analysis.

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- *Levene's (Homogeneity of Variance) Test*

Levene's test checks if variances are equal among groups. A p-value > significance level indicates approximately equal variances; < significance level suggests unequal variances. This test is crucial for parametric tests like ANOVA, which assume equal variances. Unequal variances can lead to incorrect conclusions. Levene's test helps researchers decide on appropriate statistical tests. If variances differ, non-parametric tests like Kruskall-Wallis may be used.

- *Kruskall-Wallis Test*

The Kruskal-Wallis test is used for independent groups when the dependent variable is ordinal or continuous but not normally distributed. It compares medians across groups, and a significant result suggests at least one group differs. It doesn't specify which groups differ; post-hoc tests are needed for that. If p < significance level, there are significant differences among groups. If p > significance level, there's insufficient evidence to conclude differences.

- *Pairwise Comparison of Treatment*

Pairwise comparison is a method to compare means or medians of multiple groups. It examines every pair of groups to identify significant differences. This method is used after an omnibus test (e.g., Kruskal-Wallis) to specify which groups differ. If p ≤ significance level, the difference is significant; if p > significance level, the difference is not significant.

## III. RESULTS

➢ *Organoleptic Evaluation of the Ginger Extract*

The extract exhibited a brown color and had a consistency of an oily syrup. Its aroma profile was distinctly gingery, characterized with a strong intensity of odor.

➢ *Determination of Zones of Inhibition (ZOI)*

The effectiveness of a specific antibiotic or extract is demonstrated by the presence of clear zones of growth inhibition on the culture plate. These zones, known as ZOIs, encircle the disk containing the antimicrobial substance. Measurement of ZOI diameter was done using a ruler in millimeters. It's important to understand that the selection of chemotherapeutic medications isn't solely based on the antibiotic with the largest ZOI as noted by Kreger et al. (1980). This is due to various factors affecting ZOI size, including the density or viscosity of the culture medium, the diffusion rate of the antibiotic, and its concentration.

All of the disks in 3 replicates containing different concentrations of ginger exhibited zones of inhibition. GC50 with 50% ginger concentration exhibited 26 mm in all 3 replicates. GC 75 with 75% ginger concentration exhibited 28 mm in replicate 1, 30 mm in replicate 2, and 26 mm in replicate 3. GC100 with 100% ginger concentration exhibited 26 mm in replicate 1, 32 mm in replicate 2, and 34 mm in replicate 3, as shown in Table 2.

Table 2 Zones of inhibition of the disks containing the combination of Ginger (G) and Clindamycin (C) in 3 replicates against the S. aureus using Kirby-Bauer Test

| Concentration | Replicate | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| GC50 | 26 mm | 26 mm | 26 mm |
| GC75 | 28 mm | 30 mm | 26 mm |
| GC100 | 26 mm | 32 mm | 34 mm |

Results show in Table 3 that all of the disks in 3 replicates containing pure ginger (G) and pure clindamycin (C) exhibited zones of inhibition. Ginger (G) exhibited 4mm in replicates 1 and 2 and 6mm in replicate 3. clindamycin exhibited 34 mm in 3 replicates.

Table 3 Zones of inhibition of the control agents, Ginger (G) and Clindamycin (C) in 3 replicates against the S. aureus using Kirby-Bauer Test

| Control Agents | Replicate | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| G | 4 mm | 4 mm | 6 mm |
| C | 34 mm | 34 mm | 34 mm |

➢ *Synergy Determination of Mean Zones of Inhibition using Kirby-Bauer Test*

Results on the mean zones of inhibition of the different concentrations seen in Figure 1 and Table 4 revealed that GC50 exhibited a mean ZOI of 26 mm of the 3 replicates. GC75 exhibited a mean ZOI of 28 mm while GC100 had a mean ZOI of 30 mm. Out of the 3 concentrations, GC100 with 100% concentration of ginger exhibited the greatest mean ZOI. The control agents' mean ZOI were also calculated for comparison. Ginger exhibited a mean ZOI of 4 mm while

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clindamycin had a mean ZOI of 34 mm. S. aureus is considered susceptible to clindamycin with a zone of inhibition greater than 21 mm in diameter as according to Clinical and Laboratory Standard Institute (2020).

A synergistic effect is when the combined effect of ginger extract and clindamycin is greater or more potent than the sum of the individual effects of Ginger and clindamycin (Chanda and Rakholiya, 2011). The sum of the individual effects of Ginger (4.67mm) and clindamycin (34mm) is 38.67mm. This means that in order to have a synergistic effect, the extract-antibiotic combination should have a mean ZOI that is greater than 38.67mm. Results showed that none of the concentration exhibited a synergistic effect based on their mean ZOI.

The descending order of mean ranks from GC100 (10.17), GC75 (8.33), and GC50 (6.00) underscores a dose-response relationship, where higher concentrations of treatment correspond to better outcomes. This trend aligns with previous studies suggesting higher doses often improve effects (Kosnicki et al., 2019). The pattern in mean ranks indicates that as the concentration of GC increases, the outcomes improve. GC100, having the highest mean rank, demonstrates the most significant positive effect, followed by GC75 and then GC50, showing a progressive decrease in effectiveness with lower concentrations.

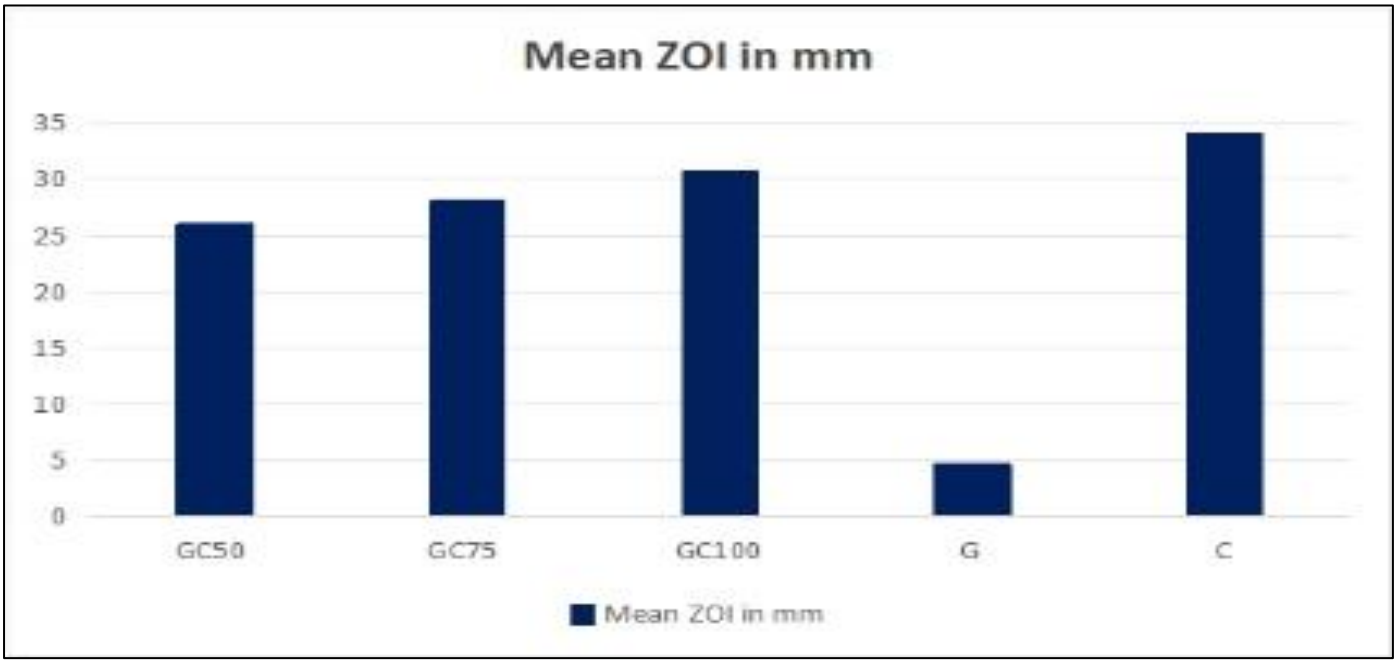


Fig 1 Mean Zones of Inhibition of the Extract-Antibiotic Combination in Different Concentrations and Control Agents

Table 4 ZOI and Mean ZOI of the Extract-Antibiotic Combination in Different Concentrations and Control Agents

| Concentration | Replicate | | | Mean ZOI | ±SD | Mean Rank |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | |
| GC50 | 26 mm | 26 mm | 26 mm | **26 mm** | **±0.00** | **6.00** |
| GC75 | 28 mm | 30 mm | 26 mm | **28 mm** | **±2.00** | **8.33** |
| GC100 | 26 mm | 32 mm | 34 mm | **30.67 mm** | **±4.16** | **10.17** |
| **Control Agents** | | | | | | |
| G | 4 mm | 4 mm | 6 mm | **4.67 mm** | **±1.15** | **2.00** |
| C | 34 mm | 34 mm | 34 mm | **34 mm** | **±0.00** | **13.50** |

- *Synergy Determination of Antibiotic-Extract Combination using Cooperative Effect Synergy*

The concept of synergistic interaction, where one antimicrobial agent enhances the effectiveness of another, prompting researchers to explore the synergistic activity of plant-derived compounds and traditional antibiotics. Utilizing Geary's model (2013), researchers initially evaluated the synergy profile. This method of determining the synergy is very much recommended. It lacks any additional matrix which is advantageous, as it obviates the need for an axiomatic mathematical theory for the addition of dose-effect curves and the complications that come along with it. Furthermore, the cooperative effect synergy met the criteria recently adopted by the US Food and Drug Administration

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

(FDA, 2003) for the assessment of combination therapies; i.e., "two or more drugs may be combined in a single dosage to enhance the safety or effectiveness of the principal active component."

Table 5 shows the synergy profile of the different combinations and concentrations tested by comparing the mean zones of inhibition to the mean ZOI of the control agents alone. All of the combinations appeared to be antagonistic since the value of their mean ZOI is lesser than the mean ZOI of clindamycin. It is important to note that despite the "Antagonistic" remark of the combination based on the standards of synergy from the Cooperative Effect Synergy model, it does not automatically mean that such combination cannot be employed for treatment uses. It should be noted that in cooperative effect synergy, the basis on denoting that the combination is synergistic is solely upon the comparison of the zones of inhibition values.

Table 5 Synergy Profile of the different concentrations using Cooperative Effect Synergy

| | Mean ZOI | Cooperative Effect Synergy |
|---|---|---|
| GC50 | 26 mm | Antagonistic |
| GC75 | 28 mm | Antagonistic |
| GC100 | 30.67 mm | Antagonistic |

➢ *Results of the Statistical Treatment of Data*

A Shapiro-Wilk test shown in Table 6 was conducted to assess the normality of the inhibition scores. The results indicated a significant deviation from normality, (W = 0.880, p = 0.048). The test statistic $W$ is reported as 0.880. This value represents the degree of agreement between the sample data (inhibition scores) and the expected values under the assumption of a normal distribution. A $W$ value close to 1 indicates a better fit to the normal distribution, while smaller values suggest deviations from normality. In this case, $W$ = 0.880 indicates some departure from normality.

The p-value associated with the Shapiro-Wilk test is reported as 0.048. In this case, $p$ = 0.048 indicates that there is a 4.8% probability of observing a test statistic as extreme as $W$ =0.880 under the assumption of normality. This low p-value suggests a violation of the assumption of normality for the inhibition scores prompting alternative non-parametric tests to be considered.

Table 6 Shapiro-Wilk (Normality Test) Results

| | W | p |
|---|---|---|
| **Inhibition** | 0.880 | 0.048 |
| Note: A low p-value suggests a violation of the assumption of normality | | *Significant at 0.05 level* |

A Levene's test was performed to evaluate the homogeneity of variances for the inhibition scores across different groups. The test statistic $F$ is reported as 5.72. The degrees of freedom are presented as (4,10). The first value (4) represents the degrees of freedom for the numerator. The second value (10) represents the degrees of freedom for the denominator. The p-value associated with Levene's test is reported as 0.012.

The test results were significant, (F (4,10) = 5.72, p = 0.012), indicating that the variances are not equal across groups. This suggests a violation of the assumption of homogeneity of variances. Because of this, the Kruskal-Wallis test was considered.

The test statistic was reported as $\chi 2$ (4) = 11.9 with the degrees of freedom of 4. The p-value associated with the Kruskal-Wallis test seen in Table 7 was reported as 0.018. This p-value indicates the probability of observing a test statistic as extreme as $\chi 2$ (4) = 11.9 $\chi$ under the null hypothesis of no difference between groups.

Table 7 Kruskall-Wallis Test Results

| | $x^2$ | df | p |
|---|---|---|---|
| **Inhibition** | 11.9 | 4 | 0.018 |

Since the p-value (0.018) is less than the significance level of 0.05, this suggests that there are statistically significant differences in inhibition scores among the groups. This means at least one group has an inhibition score that is different from the other groups. If a specific group has a different median inhibition score than the others, it suggests that there are specific characteristics or factors associated

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

with that group that influence inhibition levels differently compared to the other groups.

Subsequent pairwise comparisons indicated that the inhibition scores differed significantly between several group pairs. Specifically, significant differences were found between Ginger vs. GC100 (Test Statistic = 2.301, p = 0.021), Ginger vs. clindamycin (Test Statistic = -3.240, p = 0.001), and GC50 vs. clindamycin (Test Statistic = -2.113, p = 0.035). These results suggest that these groups have statistically different inhibition scores which means that there is a significant variation in the inhibition scores between these groups.

Significant difference was found in Ginger vs. GC100 (Test Statistic = 2.301, p = 0.021). This suggests Ginger alone has lower inhibition scores than the GC100 combination. This aligns with the study of Fahrinda et al. (2018) where combination of extract and antibiotic showed greater effectivity compared to that of extract alone.

A significant difference was found in Ginger vs. clindamycin (Test Statistic = -3.240, p = 0.001). This suggests that the commercial antibiotic greatly possesses a higher effectiveness than the plant extract which further verifies the clinical efficacy of commercial antibiotics.

In the extract-antibiotic combination, only GC50 revealed a significant difference to clindamycin. This means that there is statistical variation in comparison with the two groups wherein the inhibition scores of GC50 is significantly lower than that of clindamycin. While lower concentrations may have some impact, they do not reach the effectiveness of the positive group. This finding aligns with literature indicating that higher dosages often yield better therapeutic outcomes (Frizelle et al., 2018). Both GC75 and GC100 showed that there is no significant difference in the inhibition scores when compared to clindamycin. However, GC75 (p-value: 0.145) is closer to 0.05 than GC100 (p-value: 0.348) which suggests a higher likelihood of being significantly different than GC100. In GC100, the p-value is much higher and suggests a lower likelihood that there is a significant difference in its inhibition score compared with clindamycin. This means that GC100 has exhibited an inhibition that is nearer to that of clindamycin than GC75.

Results suggest that out of all 3 concentrations, GC100 poses a greater inhibition score. Although it did not exhibit synergistic quality, it still demonstrates a significant antimicrobial effect that merits further investigation as a potential alternative or adjunctive treatment, as shown in Table 8.

Table 8 Pairwise Comparison of Treatment of all Data

| Comparison | Test Statistic | P-value | Remarks |
|---|---|---|---|
| Ginger vs. GC50 | 1.127 | 0.260 | Not significant |
| Ginger vs. GC75 | 1.785 | 0.074 | Not significant |
| Ginger vs. GC100 | 2.301* | 0.021 | Significant |
| Ginger vs. Clindamycin | -3.240*** | 0.001 | Significant |
| GC50 vs. GC75 | -0.675 | 0.511 | Not significant |
| GC50 vs. GC100 | -1.174 | 0.240 | Not significant |
| GC50 vs. Clindamycin | -2.113* | 0.035 | Significant |
| GC75 vs. GC100 | -0.517 | 0.605 | Not significant |
| GC75 vs. Clindamycin | -1.456 | 0.145 | Not significant |
| GC100 vs. Clindamycin | -0.939 | 0.348 | Not significant |

Note: ****significant at 0.01 level* **significant at 0.05 level*

## IV. DISCUSSION

Setting the foundation for additional study, the organoleptic assessment of the ginger extract revealed a brown oily syrup with a strong ginger scent. The extract's potential antibacterial capabilities were highlighted by the observation of antagonistic behavior across all tested concentrations, as shown by their Mean Zone of Inhibition (ZOI) in the Kirby-Bauer test. The Cooperative Effect Synergy testing, which revealed antagonism at all concentrations, supported this observation even more. Nevertheless, statistical analysis revealed a noteworthy exception among these findings. The 100% Ginger Concentration with Clindamycin (GC100) was the most notable of the three concentrations tested; its outcomes were closer to those of clindamycin alone. This distinction highlights the potential for ginger extract and clindamycin to

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25mar816

work synergistically, opening up a potentially new direction for further study and application in antimicrobial medicines.

As the study progresses, there are several areas worth exploring for future research. One avenue is to investigate different extract forms of ginger. This could involve exploring the efficacy of various forms such as aqueous or other solvent-based extracts, in combination with clindamycin, to determine which form yields the most effective results. Another area to explore is the different methods of drying ginger, including freeze-drying, sun drying, and oven drying. This exploration aims to optimize the yield of bioactive compounds and enhance the antimicrobial properties of ginger extracts. It is advisable to conduct the study as soon as possible to ensure the efficiency of all materials, including the extract. Additionally, exploring the synergistic potential of ginger extract with other antibiotics besides clindamycin could uncover alternative combinations that may be more effective. Testing the antibacterial activity of ginger extract and clindamycin combination against bacterial species other than S. aureus would broaden the scope of the research. Furthermore, considering in vivo studies to assess the combination's efficacy in a more complex biological system may reveal synergistic effects not observed in vitro.

## V. CONCLUSION

The interaction between ginger extract and clindamycin against Staphylococcus aureus has been found to exhibit antagonism, as demonstrated by standard susceptibility testing methods. This antagonistic effect means that the combination is less effective than either agent alone in inhibiting bacterial growth. Despite this, interestingly, at a 100% concentration, the combination of ginger and clindamycin shows antibacterial activity that is comparable to that of clindamycin used alone.

This observation suggests that, under specific conditions, the high concentration of the combination might still offer therapeutic benefits. This potential could be particularly valuable in scenarios where resistance to standard antibiotics is a concern or where a natural product like ginger is preferred for its additional health benefits or lower side effect profile.

However, the presence of antagonism at other concentrations highlights the complexity of interactions between plant extracts and antibiotics. It underscores the necessity for careful consideration and optimization of dosage to avoid reducing the efficacy of treatment. Therefore, more comprehensive research is required to fully understand the conditions under which the combination of ginger and clindamycin can be effectively used.

In conclusion, while the combination of ginger extract and clindamycin shows antagonism against S. aureus, the 100% concentration of the combination holds promise as an alternative treatment. Its comparable effect to clindamycin alone indicates potential therapeutic value, which warrants further investigation to maximize its benefits in clinical applications.

## ACKNOWLEDGEMENT

This study owes its success to the generous support and collaboration of many individuals. We acknowledge the divine presence of God for His grace, guidance, and blessings. Our heartfelt gratitude goes to our families for their unwavering love, support, and sacrifices, which have been our source of strength and inspiration.

Special thanks to Bryle Dale G. Maano, Scott L. Gahum, and Alberto T. Ramirez Jr. for their knowledge and guidance. We are sincerely grateful to our research teacher, Junge B. Guillena, PhD, for his invaluable mentorship and support, and to our research adviser, Ma'am Cath Joy B. Ronquillo, for her insightful feedback and encouragement throughout this journey.

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