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Research Article

Study of the Efficacy of Concentrated Alcohol-Based Hand Sanitizer in Preventing Transmission of Pathogen

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ABSTRACT

An integral part of the human body is the hands, which most frequently come into contact with various items; they can be an intermediary for spreading infections from hands to food and potentially eaten by humans. Maintaining hand hygiene is the right strategy to avoid this spread, which includes using an alcohol-based hand sanitizer. This study examines the potential for varying concentrations of alcohol-based hand sanitizer to transmit pathogens through the palms. The form of research is a laboratory experiment with an appropriate research design. Test the antibacterial potential of variations in alcohol-based hand sanitizer concentrations of 40, 50, 60, 70, and 70% using well diffusion and dilution methods. The tests were done in quadruplicates. The test bacteria used are Escherichia coli, Enterotoxigenic Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus. The results of the research showed that of the four variations in the concentration of alcohol-based hand sanitizer, the bacteriostatic ability against Salmonella typhimurium, Pseudomonas aeruginosa, and Klebsiella pneumoniae bacteria was at a concentration of 40% to 80%. The bactericidal activity at a concentration of 80% was demonstrated by Escherichia coli, Enterotoxigenic Escherichia coli, and Staphylococcus aureus. Bactericidal activity was demonstrated against Escherichia coli, Enterotoxigenic Escherichia coli, and Staphylococcus aureus at an 80% concentration.

Key words: *Antibacterial efficacy; Alcohol-based hand sanitizer; Transmission of pathogen; Palms.*

Introduction

Hands are a haven for microorganisms, particularly bacteria. As the body parts that frequently come into contact with various objects, hands can transfer pathogens from surfaces to food, leading to potential ingestion by humans [1–4]. Good hand hygiene can prevent the spread of microorganisms and reduce the frequency of hospital-acquired infections [5–8]. Pathogenic bacteria with the highest frequency levels cause diseases in humans by being transmitted through hand contact, including *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella* sp. [3], [9–12].

Staphylococcus aureus is a widespread pathogenic bacterium that frequently infects the human body, living on the skin, sweat glands, and the digestive tract. [13–15]. These bacteria in bones and joints can cause osteomyelitis and septic arthritis; in respiratory organs, they can cause pneumonia; and in cardiovascular organs, they can cause infective endocarditis [12],[16]. *Klebsiella pneumoniae* is a pathogenic bacteria that can cause pneumonia and urinary tract infections in humans. This bacteria is the leading cause of nosocomial infections around the world. Children and people with a weaker immune system, such as patients in the ICU, patients with cancer, patients with HIV, patients on chemotherapy, and patients with diabetes mellitus, are especially vulnerable to these bacterial infections [11],[17]. Besides *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* is a source of nosocomial infections, including pneumonia and urinary tract infections. These bacteria have a natural resistance mechanism against many antibiotics and disinfectants [18]. *Escherichia coli* and *Salmonella typhimurium* are pathogenic bacteria that mainly cause food-borne diseases transferred and spread by hand. *Salmonella typhimurium* and *Escherichia coli* can cause gastroenteritis infection [19],[20].

To effectively prevent the transmission of pathogens, it is crucial to maintain proper hand hygiene. this involves washing hands with soap and water when dirty or using a hand sanitizer containing alcohol when hands do not appear visibly contaminated [9],[21–23]. Washing your hands every time is often difficult, so using hand sanitizer is a more practical solution for cleaning your hands [24],[25]. Hand hygiene is essential, but it is difficult to do in every condition, this is because we don't always use running water and antiseptic soap [14],[26]. Using hand sanitizers as an alternative hand-cleaning fluid is one of the important requirements for maintaining hand hygiene [10],[27],[28]. All types of bacteria found on the palms of the hands can be reduced with hand sanitizer [29],[30]. Several studies have shown that hand sanitizer is useful in lowering the occurrence of digestive diseases, the number of absent from school among students, and the number of illnesses in some students, as well as reducing the transmission of disease within the household [29],[31],[32].

The most popular formulations of various commercial hand sanitizer products in great demand are those based on alcohol as the active ingredient [33],[34]. Due to its extensive antibacterial activity against a variety of pathogens, the World Health Organization strongly recommends the use of alcohol as a primary component in hand sanitizers as the "*gold standard*" for hand disinfection [32],[35],[36]. Hand sanitizer based on alcohol has been shown to protect hands from a variety of bacteria. The WHO recommends using alcohol as a basic ingredient in hand sanitizers [34]. Rubbing your hands with alcohol for 20-30 seconds has been proven to eliminate 99% of bacteria on your hands [32],[37]. The alcohol concentration in hand sanitizers must be carefully evaluated as it is a crucial factor in determining their effectiveness [10],[38], pH, viscosity, and hydrogen peroxide content in hand sanitizer composition are other parameters related to product functionality and acceptance by users [39],[40]. Based on the reasons mentioned above, it is necessary to carry out research to examine the antibacterial properties of various concentrations of alcohol as a new raw material for hand sanitizer.

Materials and methods

Materials

The tools used in this research include stir rods, volume pipettes, 50 ml measuring flasks, Erlenmeyer, Petri dishes, steel pipettes, tweezers, 1000 µl micropipettes, 100 µl micropipettes, blue tip, yellow tip, vernier caliper, incubator, autoclave, Biosafety Cabinet class II. The

materials needed in this research include 96% ethanol, glycerin, distilled water, PZ, and Mueller Hinton Agar (MHA) medium. The test bacteria used include *Escherichia coli, Enterotoxigenic Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus.*

Methods

1. *Preparation of Various Alcohol Concentrations in Hand Sanitizer*

Prepare 96% ethanol, glycerin, and distilled water, then adjust the alcohol concentration as the basic ingredient for hand sanitizer to 40%, 50%, 60%, 70%, and 80%. We measured each concentration up to 50 mL. Combine 20.8 ml of 96% ethanol with 8.3 ml of glycerin to achieve a 40% concentration. Combine 26.0 mL of 96% ethanol with 8.3 mL of glycerin to achieve a 50 mL concentration. A 60% concentration comprises 31.3 ml of 96% ethanol and 8.3 ml of glycerin. 36.5 ml of 96% ethanol and 8.3 ml of glycerin generate a 70% concentration. We prepared the 80% concentration using 41.6 ml of 96% ethanol and 8.3 ml of glycerin. We transferred the mixture into a 50-ml measuring flask, added distilled water until it reached the specified level, and then thoroughly mixed it.

2. *Antibacterial Efficacy Test*

The microbiology laboratory of the Faculty of Health Science, Universitas Ma'arif Hasyim Latif, Sidoarjo, East Java, investigated the antimicrobial efficiency of various alcohol concentrations as a base hand sanitizer.

3. *Test Bacteria*

The antibacterial efficacy test was carried out in the Faculty of Health Science Universitas Ma'arif Hasyim Latif, Sidoarjo, East Java, Microbiology laboratory, kindly provide clinical isolates of bacteria like *Escherichia coli,* Enterotoxigenic *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Staphylococcus aureus*.

4. *Confirmation of the Test Bacteria*

Biochemical identification and Gram staining were carried out to confirm the test bacteria. We inoculated the bacteria *Escherichia coli,* Enterotoxigenic *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* into nutrient broth fertilizer medium. We placed them in an incubator at 37 °C for 24 hours in the presence of oxygen. From the fertilizer medium, it is then subcultured on Eosin Methylene Blue Agar (EMBA), Blood Agar Plate (BAP), McConkey Agar (MCA), Endo Agar (EA), and Violet Red Bile Agar (VRBA). Especially for isolates of *Salmonella typhimurium* added by subculture on *Salmonella Shigella Agar* (SSA), Hektoen *Enteric Agar* (HEA), and *Xylose Lysin Deoxycholate Agar* (XLDA) medium, all medium were incubated at 37 °C for 24 hours in aerobic conditions. We conducted biochemical tests by subculturing them in various biochemical reaction medium [19],[32],[41]. *Staphylococcus aureus* bacteria were inoculated in NaCl broth fertilization medium and incubated aerobically at 37 °C for 24 hours. The fertilizer medium were subcultured on MSA and BAP medium and were then incubated at 37 °C for 24 hours in aerobic conditions [42]. We isolated the test bacteria, stored them in a storage medium at a temperature of 2–8 °C, and used them as needed. We standardized each test bacterium using the McFarland 0.5 standard [2],[41]. The Mc Farland 0.5 turbidity is made from a mixture of sulfuric acid (H2SO4) and barium chloride (BaCl2.2H2O) with confirmation of the accuracy of the mixed absorption density (0.08-0.10) via spectrophotometer at a wavelength of 625 nm [32]. The turbidity of the tested bacterial suspension should have been equivalent to 1.5 x 10^8 cfu/ml [43].

Table 1. Zone of Inhibition for Various Concentrations Alcohol of Hand Sanitizer Against Test Bacteria

Various Concentrations (% v/v)	Mean Zones of Inhibition (± SD)					
	Escherichia coli	**Enteroxigenic *Escherichia coli***	***Salmonella typhimurium***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Staphylococcus aureus***
40	21.68±0.81	21.64±1.10	12.98±0,25	10.60±0.39	8.80±0.30	10.33±0.46
50	24.54±0.29	23.44±1.20	14.33±0.41	11.87±0.10	9.67±0.49	11.12±0.36
60	24.46±1.06	24.85±0.42	18.19±0.48	12.21±0.23	10.55±0.24	14.60±0.68
70	30.14±0.76	26.84±0.58	19.80±0.43	13.39±0.18	11.48±0.71	22.04±0.35
80	32.08±0.20	31.73±1.47	21.98±0.43	19.20±0.40	14.33±0.66	22.37±1.08
+	63.98±0.20	63.98±0.20	29.07±0.39	20.65±0.13	32.47±0.24	32.91±0.72
–	0±0	0±0	0±0	0±0	0±0	0±0

Notes: (+) Positive Control, (–) Negative Control. Inhibitory Response: Strong (> 20 mm); Moderate (16-20 mm); Weak (10-15 mm); Less effective (< 10 mm) [49].

5. *The Study Examined the Antibacterial Activity of Alcohol-Based Hand Sanitizers Using Agar Well Diffusion Methods*

We used this method to investigate the test organisms' susceptibility to various alcohol concentrations in hand sanitizer. Each sample's agar well diffusion was repeated quadruplicated for each sample. 5 ml of MHA is placed onto empty sterile Petri dishes and let to solidify (as a based layer), the 4 sterile fertilizer cylinders are placed on it and the distance between cylinders is regulated. After homogenizing a suspension of 1 ml of test bacteria, positive control (96% alcohol), and negative control (glycerin) with 20 ml of MHA in a tube, we placed the mixture onto Petri dishes containing sterile steel cylinders as the second layer (seed layer) and allowed it to solidify. Once solidified, the steel cylinder backer is aseptically removed from the Petri dish to form a good hole. In each filled with controls or supplies of hand sanitizer with variations in concentration, the Petri dish is incubated for 18-24 hours at 370C. There is an inhibition zone formed around the well, its area is measured using a caliper [43],[44].

6. *Dilution Methods for Testing the Antibacterial Activity of an Alcohol-Based Hand Sanitizer*

We used the nutrient broth for each concentration of hand sanitizer to determine the minimum concentration necessary to inhibit the growth of a specific test organism in vitro. The establishment of the Minimum Inhibitory Concentration (MIC) was accomplished through the utilization of the broth dilution method [32],[41],[43], by preparing several alcohol concentrations. Afterward, one milliliter of hand sanitizer with different concentrations was added to a tube containing the same volume (1 ml) of nutrient broth that had been inoculated with a standardized test organism. This resulted in final concentrations of alcohol-based hand sanitizer at 40%, 50%, 60%, 70%, and 80%.

A tube containing nutrient broth and bacteria without sanitizer, and another containing sanitizer and broth without bacteria served as the negative and positive controls, respectively. Each experiment is replicated in quadruplicate. Ultimately, we incubated the tubes for 18–24 hours to assess and quantify the presence of visible growth (turbidity). The MIC refers to the concentration of different alcohols in hand sanitizers where no visible growth was observed in the control group [32],[45].

We conducted the MBC test using the pour-plate technique. We prepared seven aseptic and empty Petri dishes, along with thawed Mueller Hinton Agar. Hand sanitizer based on various alcohol concentrations, positive control (96% alcohol), and negative control (*glycerin*) which have been added with test bacteria, 1 ml each taken and put into a Petri dish. The Petri dish was filled with 15 ml of MHA, mixed the mixture is then left undisturbed until it hardened (about 15 minutes). The dishes were incubated at 370C, 18-24 h. The observation was made about bacterial colonies' growth or lack thereof on MHA. The bacterial colony count was determined using a colony counter. The MBC was determined by observing the absence of bacterial colonies on the MHA, or by achieving a 99.9% reduction in bacterial growth compared to the initial inoculum on the subculture [45–48]. The experiments were conducted in quadruplicates.

7. Controlling and Ensuring Data Quality

To ensure rigorous research standards were upheld throughout the study, we meticulously adhered to aseptic techniques to prevent any potential contamination. All experimental procedures were executed in quadruplicate, allowing for robust replication and enhancing the reliability of our findings. To further validate the sterility of our experimental setup, we subjected all prepared medium to an overnight incubation period without the introduction of any microorganisms. This step was crucial to monitor and confirm the absence of any unintended microbial growth, which would indicate contamination. Additionally, we assessed the capability of the medium to adequately support microbial growth by inoculating them with known control strains.

8. The Data Evaluation and Interpretation

The data was meticulously collected, examined, and presented employing suitable statistical methodologies. The data was analyzed, and the findings are reported as the mean ± SD. SPSS program version 26 was used for statistical analysis.

Results and Discussion

These results confirmed the test microbes' credentials using a variety of biochemical techniques. We assessed the antibacterial efficacy by measuring the zone of inhibition against the specified test bacterium. We present the following data on the antibacterial efficacy of alcohol-based hand sanitizer.

Table 1 provides a comprehensive overview of the variations in alcohol concentration used as a primary ingredient in hand sanitizers and the corresponding antibacterial effectiveness against a range of tested bacteria. The study explored the antibacterial activity of hand sanitizers formulated with different alcohol concentrations, specifically focusing on solutions with alcohol content ranging from 40% to 80% by volume. The research categorized the inhibitory responses based on the effectiveness of each concentration in preventing bacterial growth, with categories ranging from less effective to strong. This systematic approach allowed for a clearer understanding of how different alcohol levels impact various bacterial species.

The data revealed that a 40% alcohol-based hand sanitizer displayed strong antibacterial activity against *Escherichia coli* and Enterotoxigenic *Escherichia*

coli, placing these bacteria in the strong inhibition category (Table 1). This indicates that even at a lower alcohol concentration, certain bacteria can be effectively targeted and controlled. On the other hand, the same 40% concentration was found to exert only moderate inhibitory effects on *Salmonella typhimurium* and *Pseudomonas aeruginosa*, demonstrating that not all bacteria are equally susceptible to lower alcohol concentrations. *Klebsiella pneumoniae* showed a more resistant profile, where alcohol concentrations of 40% and 50% resulted in weak inhibition, suggesting that these levels are insufficient for robust bacterial control. However, increasing the concentration to between 60% and 80% shifted the inhibitory effect to the moderate category, highlighting the concentration-dependent nature of alcohol's antibacterial activity.

In addition, the study examined the effect of alcohol concentration on *Staphylococcus aureus*, a common skin pathogen. It was observed that concentrations of 40% to 60% were only weakly effective in inhibiting this bacterium. In contrast, when the alcohol concentration was increased to 70% and 80%, the inhibition of *Staphylococcus aureus* improved significantly, moving into the strong category (Figure 2). This underscores the necessity of higher alcohol concentrations for effective control of certain more resistant bacterial strains. Overall, the findings suggest that selecting the appropriate alcohol concentration in hand sanitizers is crucial for maximizing their antibacterial effectiveness, especially in situations where a broad spectrum of bacterial resistance is encountered.

Based on Figure 1, the inhibition test of alcohol-based hand sanitizer at 80% concentration against *Escherichia coli*, Enterotoxigenic *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* produced zones of inhibition. The inhibition zone produced against Escherichia coli had a better inhibition zone in giving 80% concentration. The clear zone formed is due to the activity of alcohol acting as an antibacterial. Bacterial growth can be inhibited because the bacterial cell surface experiences a decrease in voltage so that cell permeability increases which will cause cell leakage so that intracellular compounds will come out which can cause death.

The one-way ANOVA statistical test resulted in a significance value 0,000, using an α=0,05. This shows significant differences in alcohol concentration among the five tested microorganisms in hand sanitizers. For *Klebsiella pneumoniae*, we used the Kruskal-Wallis test, which resulted in a significance value of 0,000, lower than the predetermined threshold of 0,05. This indicates significant differences in the effects of various treatments and the concentration of alcohol-based hand sanitizer. Tukey statistical test results show significant differences between variations in alcohol concentration on hand sanitizers against *Escherichia coli, Salmonella typhimurium*, and *Pseudomonas aeruginosa.* In Enterotoxigenic *Escherichia coli* there is a significant difference between 40% and 50 to 80% alcohol concentration, at 50% alcohol concentration, there is no significant difference between 60% and 70%. On *Staphylococcus aureus* there was no real difference in various alcohol concentrations of 70 and 80%. According to the results of the Tukey test, there were notable variations in concentrations of alcohol-based hand sanitizers between the treatments for *Klebsiella pneumoniae*.

Data analysis was continued with the objective of Principal Component Analysis (PCA) to reduce the dimensions of data without significantly reducing the characteristics of the data. Based on Table 2, the initial eigenvalues show the number of variables, namely: Alcohol-Based Hand Sanitizer: 5,686 + 0,235 + 0,055 + 0,019 + 0,003 + 0,001. By eigenvalues, there is one variation factor, namely 5.686. Based on this, 1 component can be formed from the 6 variables analyzed where the eigenvalue is

greater than 1. Component 1 is the component that can explain 94.774% of the variation. Furthermore, determining the number of components in PCA can be displayed visually via a display *Scree plot* (Figure 2). A scree plot is a graphic used in *Principal Component Analysis* (PCA) to help determine how many principal components to retain in the analysis.

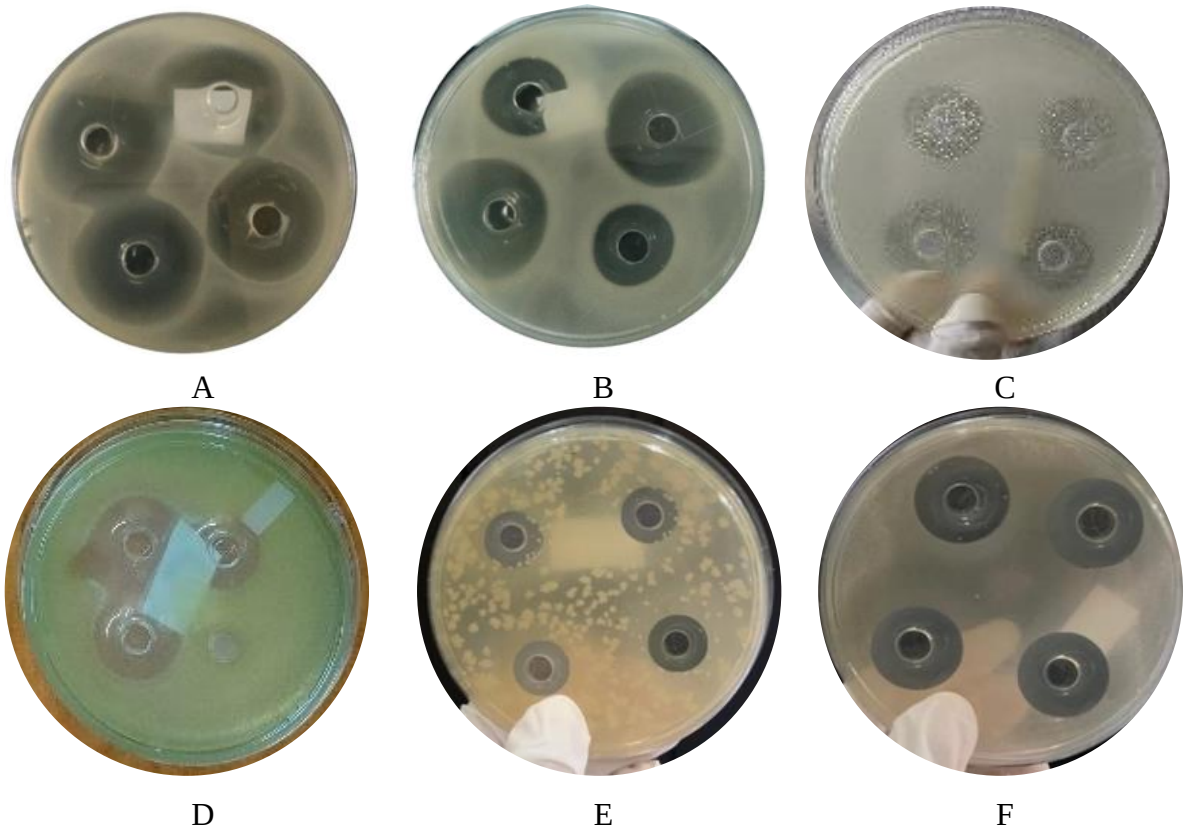

Figure 1. Test the inhibitory of alcohol-based hand sanitizer at a concentration of 80% against the test bacteria: A. *Escherichia coli;* B. Enterotoxigenic *Escherichia coli;* C. *Salmonella typhimurium*; D. *Pseudomonas aeruginosa;* E. *Klebsiella pneumoniae;* and F. *Staphylococcus aureus.*

Based on Figure 2, it is evident from the scree plot that component number 1 has an eigenvalue greater than 1, indicating that it captures a significant portion of the variance in the dataset, specifically regarding the effectiveness of alcohol-based hand sanitizers. In principal component analysis (PCA), an eigenvalue exceeding 1 suggests that this component is highly influential in explaining the variability in data, particularly in relation to the sanitizer's ability to inhibit bacterial growth. The findings reveal that this component represents the overall effectiveness of the sanitizers against various bacteria, with a marked emphasis on *Escherichia coli*, which appears to be the most significantly inhibited among the six bacteria tested. This strong inhibitory effect against *Escherichia coli* may be due to the bacterium's specific susceptibility to the bactericidal properties of alcohol, which can disrupt its cellular processes more effectively than others. The eigenvalue's magnitude suggests a non-random, consistent factor influencing the inhibition patterns observed, likely linked to the alcohol concentration and composition of the sanitizers.

Table 2. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Vari-ance	Cumulative %	Total	% of Vari-ance	Cumulative %
1	5.686	94.774	94.774	5.686	94.774	94.774
2	.235	3.911	98.686			
3	.055	.920	99.606			
4	.019	.322	99.928			
5	.003	.052	99.981			
6	.001	.019	100.000			

Extraction Method: Principal Component Analysis.

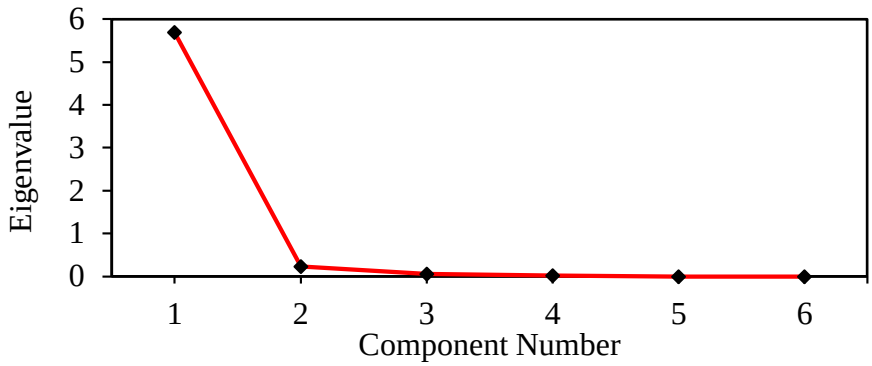

Figure 2. Scree Plot PCA.

The study focuses on evaluating the effectiveness of alcohol-based hand sanitizers formulated according to World Health Organization (WHO) recommendations. These guidelines specify the inclusion of ingredients like hydrogen peroxide for its disinfectant properties, ethanol for its germicidal action, and glycerin to moisturize the skin. The research examines sanitizers with varying alcohol concentrations of 80%, 70%, 60%, 50%, and 40% by volume (v/v) (Table 3), to determine the most effective formulation for killing germs and preventing infections while being safe for regular use.

Established provisions determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. Establish the MIC values by observing a 90% decrease in colony growth, or 1 log CFU/ml. The MBC value can be determined if no bacterial growth exists in the medium. The MIC and MBC test results can be seen in Table 3, the MIC of alcohol-based hand sanitizers for *Escherichia coli*, Enterotoxigenic *Escherichia coli* is at a concentration of 60%, for *Staphylococcus aureus* is a concentration of 70%, while for *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* at a concentration of 80%. MBC results for *Escherichia coli*, Enterotoxigenic *Escherichia coli*, and *Staphylococcus aureus* at a concentration of 80%. In contrast, for *Salmonella typhimurium Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* up to a concentration of 80% there were no MBC. We evaluated the effectiveness of the alcohol-based hand sanitizer using the well diffusion and dilution method on six (6) strains of bacteria. The findings revealed that the sanitizer was able to affect three (3) of the tested bacteria, namely *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, which are known to be difficult to inhibit or kill.

Salmonella typhimurium is a Gram-negative bacterium that is difficult to inhibit

due to its complex structure, making it more resistant to alcohol-based hand sanitizers [53],[54]. *Pseudomonas aeruginosa* is a type of bacteria that is classified as a basil-negative Gram. It can produce pigments that dissolve in water, such as pyocyanin and pyoverdine. These pigments give the bacteria their distinctive blue-green color on solid medium. *Pseudomonas aeruginosa lives in slime-enclosed biofilms, which allow it to survive and replicate in human tissues as well as on medical devices.* The biofilm assists in protecting *P. aeruginosa* antibodies and phagocytes from host production, resulting in antibiotic and antimicrobial resistance in this organism. The flexible nutritional requirements allow it to thrive in less hospitable environments, making it difficult to eradicate contaminated areas such as operating rooms, hospital rooms, clinics, and medical equipment [12],[55]. The key components necessary for *Klebsiella pneumoniae* pathogenicity include lipopolysaccharides and polysaccharide capsules. Additionally, *Klebsiella pneumoniae* is capable of forming biofilms. Fimbriae types 1 and 3 in capsule polysaccharides are significant virulence factors in *Klebsiella pneumoniae*, contributing to the development of biofilms [56].

This study found that an alcohol-based hand sanitizer concentration of 40 to 80% had bacteriostatic properties against Salmonella typhimurium, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, but bactericidal properties against *Escherichia coli*, Enterotoxigenic *Escherichia coli*, and *Staphylococcus aureus.* The formulation's principal active component, ethanol, is responsible for microorganism lethality. Because the effectiveness of alcohol depends on its concentration, accurately determining the alcohol level of alcohol-based hand sanitizer may serve as a proxy for efficacy[36],[50]. The specified tolerance for ethanol content to meet the criterion is a range of ± 5% deviation (75-85% v/v) from the declared potency (80% v/v) [32],[56].

Table 3. Mean Results of Colony Counts on Test Bacteria to Determined Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Various Concentration Based-Hand Sanitizers

Various Concentrations (%v/v)	Bacteria (Log cfu/ml)					
	Escherichia coli	**Enteroxigenic *Escherichia coli***	***Salmonella typhimurium***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Staphylococcus aureus***
40	325	400	519	597	890	590
50	277	258	385	401	763	390
60	108*	122*	212	272	497	195
70	51	59	97	125	315	52*
80	0**	0**	50*	52*	199*	0**
+	0	0	0	0	40	5
–	0	0	0	0	0	0

Notes: (+) Positive Control, (–) Negative Control, (*) MIC, (**) MBC.

Conclusions

The study results of the antibacterial efficacy of four (four) variations in the concentration of alcohol-based hand sanitizer exhibited bacteriostatic capabilities against bacteria *Salmonella typhimurium, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* at concentrations ranging from 40 ─ 80 %. Bactericidal activity was demonstrated against *Escherichia coli*, Enterotoxigenic *Escherichia coli*, and *Staphylococcus aureus* at an 80% concentration.

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