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RESEARCH ARTICLE

Quality Assessment and Antimicrobial Susceptibility of Varying Formulations of Nipa Bioethanol-Derived Gel

Jayson F Cariaga[1]*, Loreli Faye T Manzano[1], Alvin G Domingo[1], Francis A Gamboa[1], Karyl Mae D Ramos[1], Shirley C Agrupis[2]

1 Researcher, National Bioenergy Research and Innovation Center, Mariano Marcos State University, Philippines
2 Professor, Graduate School, Mariano Marcos State University, Philippines

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* **Corresponding author**.

jfcariaga@mmsu.edu.ph

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Abstract

Objectives: The objective of this study is to evaluate the antimicrobial activity of alcogel treatments derived from Nipa bioethanol against common bacterial strains and provide insights into their quality assessment for enhanced effectiveness. **Methods:** Each treatment was evaluated using the Kirby-Bauer Disc-Diffusion Susceptibility test, and Rheological tests were conducted to measure the viscosity of each treatment. **Findings:** Results showed that several treatments exhibited strong antimicrobial activity against both bacterial strains, with mean zones of inhibition values ranging from 19.06 mm to 30.38 mm. Treatment A, with a mean zone of inhibition of 30.38 mm against *E. coli*, was found to be the most effective alcogel treatment. Furthermore, the study showed that viscosity and the modification of the formulation did not significantly affect the antibacterial activity of the alcogel treatments. **Novelty:** These findings provide insights into the formulation of alcogel treatments derived from Nipa bioethanol for enhanced antimicrobial activity against common bacterial strains.

Keywords: Alcogel; Nipa bioethanol; Antimicrobial activity

1 Introduction

In recent years, the demand for effective and safe hand sanitizers has skyrocketed, primarily driven by the need for improved hygiene practices and the prevention of infectious diseases [(1)]. One prominent solution that has gained significant attention is the development of alcohol-based sanitizers [(2)]. Among them, bioethanol has emerged as a sustainable alternative to traditional ethanol due to its renewable source and reduced environmental impact [(3)]. In this context, the present study focuses on the quality assessment of Nipa bioethanol-derived alcohol gel to enhance its antimicrobial activity.

The utilization of bioethanol as the primary ingredient in alcohol-based sanitizers presents numerous advantages. Derived from renewable sources such as Nipa sap as feedstock, bioethanol offers a more sustainable and environmentally friendly alternative

to fossil fuel-based ethanol. In addition, bioethanol production contributes to the reduction of greenhouse gas emissions, making it an attractive choice for the development of sanitizing products[4]. The use of Nipa bioethanol in this study brings forth an additional advantage. Nipa, a fast-growing palm native to tropical regions, has demonstrated great potential as a biofuel feedstock due to its high sugar content and efficient conversion into ethanol. By harnessing the potential of Nipa bioethanol, the researchers aimed to assess the formulation of alcohol gel for enhanced antimicrobial activity, thereby creating a more potent and reliable hand sanitizer.

In accordance with the guidelines provided by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), hand sanitizer formulations commonly consist of various components[5]. These include one or more types of alcohol, such as ethanol, isopropanol, or propanol, along with additional ingredients, excipients, and humectants. It is crucial to adhere to the recommended alcohol content in hand sanitizers, typically ranging from 60% to 95%, as this ensures effective antimicrobial and antiseptic properties[6]. As for this study, the Nipa bioethanol-derived alcohol gel that was used has a concentration of 70%.

While WHO formulations serve as a reliable reference, certain limitations are associated with their liquid consistency[7]. These limitations include challenges in spreading the sanitizer evenly and the potential for inadequate quantities being dispensed. Consequently, gel-based hand sanitizers have gained popularity due to their advantageous viscosity, which not only enhances efficacy and performance but also facilitates convenient dispensing[8]. The use of gel formulations is particularly beneficial for on-the-go sanitation purposes, as it helps mitigate the risk of leakage. Moreover, gel sanitizers offer rapid absorption, pleasant fragrance, and a comfortable sensation upon application.

This study seeks to develop a standardized formulation for Nipa bioethanol-derived alcohol gels, addressing the current lack of established protocols specific to this alcogel type. Additionally, it aims to enhance the antimicrobial efficacy of these alcohol gels by assessing different formulations and viscosity levels. By assessing the quality of Nipa bioethanol-derived alcohol gel for improved antimicrobial activity, the researchers intend to contribute to global efforts in preventing the spread of infectious diseases and improving public health.

The implications of this research are significant across various sectors, including healthcare, food handling, and everyday hygiene practices, where the use of effective hand sanitizers is essential. The findings have the potential to make a substantial impact on the field of bioethanol-based sanitizers by providing a reliable solution to combat infectious diseases and promote better hand hygiene practices. Ultimately, this study aims to contribute to public health initiatives and offer practical benefits in the form of a standardized and effective Nipa bioethanol-derived alcohol gel.

2 Methodology

2.1 Production of 70% Nipa Bioethanol-derived Alcohol Gel

The following is a list of samples that were prepared and used in the study:

1. Sample A: 0.3 grams of carbomer were dissolved in 33.23 mL of distilled water until fully dissolved. Then, 2 drops of triethanolamine were added, followed by the addition of 116.67 mL of 90% ethyl alcohol and thorough mixing.
2. Sample B: In Sample B, 0.3 grams of CMC was mixed with 33.23 mL of distilled water until fully combined. Then, 2 drops of triethanolamine were added, followed by the incorporation of 116.67 mL of 90% ethyl alcohol.
3. Sample C: Sample C involved mixing 0.3 grams of sodium alginate with 33.23 mL of distilled water until thoroughly blended. Then, 2 drops of triethanolamine were added, and 116.67 mL of 90% ethyl alcohol was introduced into the mixture.
4. Sample D: For Sample D, a mixture of 0.15 grams of carbomer and 0.15 grams of CMC was combined with 33.23 mL of distilled water. After that, 2 drops of triethanolamine were added, and 116.67 mL of 90% ethyl alcohol was included in the solution.
5. Sample E: In Sample E, 0.15 grams of carbomer and 0.15 grams of sodium alginate were blended with 33.23 mL of distilled water. Then, 2 drops of triethanolamine were added, and 116.67 mL of 90% ethyl alcohol was incorporated into the mixture.
6. Sample F: Sample F consisted of mixing 0.15 grams of CMC and 0.15 grams of sodium alginate with 33.23 mL of distilled water. Following that, 2 drops of triethanolamine were added, and 116.67 mL of 90% ethyl alcohol was introduced into the solution.
7. Sample G: To create Sample G, 0.1 grams of carbomer was combined with 33.23 mL of distilled water. Then, 2 drops of triethanolamine and 16.67 mL of 90% ethyl alcohol were added to the mixture.

8. Sample H: Sample H involved mixing 0.5 grams of carbomer with 33.23 mL of distilled water. Subsequently, 2 drops of triethanolamine and 116.67 mL of 90% ethyl alcohol were included in the solution.
9. Sample I: In Sample I, 0.3 grams of carbomer was mixed with 32.06 mL of distilled water. Then, 2 drops of triethanolamine, 116.67 mL of 90% ethyl alcohol, and 1.17 mL of glycerol were added to the mixture.
10. Sample J: Sample J consisted of blending 0.3 grams of carbomer with 32.06 mL of distilled water. Afterward, 2 drops of triethanolamine, 116.67 mL of 90% ethyl alcohol, and 1.17 mL of fragrance were incorporated into the mixture.
11. Sample K: To prepare Sample K, 0.3 grams of carbomer was mixed with 30.89 mL of distilled water. Then, 2 drops of triethanolamine, 116.67 mL of 90% ethyl alcohol, 1.17 mL of fragrance, and 1.17 mL of glycerol were added and thoroughly combined.

All the materials used to formulate the samples were prepared in an aseptic area. The samples were mixed thoroughly using a magnetic stirrer for 30 minutes until a homogenous solution was obtained. Furthermore, the physical properties of the alcogels were determined as shown in Table 1.

Table 1. Physical Properties of the produced 70% Nipa Bioethanol-derived Alcohol Gels

Alcohol Gels	pH	Temperature	Volume (mL)	Density (g/ml)
Control	6.28	29	50	0.93
Sample A	6.27	29.4	50	0.9366
Sample B	8.38	29.3	50	0.9356
Sample C	8.06	28.6	50	0.9356
Sample D	6.81	28.3	50	0.936
Sample E	7.24	28	50	0.936
Sample F	8.15	27.4	50	0.9371
Sample G	7.46	27.8	50	0.936
Sample H	7.13	26.5	50	0.918
Sample I	6.94	27.1	50	0.9386
Sample J	6.84	26.3	50	0.9373
Sample K	6.29	27.8	50	1.0003

2.2 Rheological Behavior of the produced 70% Nipa Bioethanol-derived Alcohol Gel

In this study, researchers utilized the BDV series digital viscometer (BDV-1S-5S-8S-9S) to evaluate the relative viscosity of several alcogels. This equipment is known for its advanced features, including its user-friendly interface, high precision measurement capabilities, stable rotating speed, exceptional anti-interference ability, and a broad range of working voltages.

To obtain accurate viscosity readings, all rotors of the equipment were employed with varying speeds of rotations per minute, and data was collected over a total time of three minutes. The use of different rotational speeds allowed for the characterization of the rheological behavior of the alcogels, providing a more comprehensive understanding of their physical properties.

The BDV series digital viscometer is an excellent tool for measuring the viscosity of different substances, and its application in this study enabled researchers to identify variations in viscosity among the various alcogels. These findings contribute to the development of a more in-depth understanding of the properties and behavior of alcogels, providing valuable information for various industrial, pharmaceutical, and biomedical applications.

2.3 Antimicrobial Assay using Disc-Diffusion Susceptibility Test

1. Preparation of bacterial strains. The bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) used in this study were obtained and grown in a suitable culture medium until they reach the desired density (usually around 0.5 McFarland turbidity standard). The bacterial strains were stored at 4°C until needed.
2. Preparation of antimicrobial compounds. The antimicrobial compounds to be tested were prepared as per their respective formulations. In this study, the researchers determined the antimicrobial activity of alcogels containing different hydrogels. The alcogels were prepared by mixing the hydrogels with alcohol, water, and other necessary additives, as per the formulations mentioned in the materials and methods section.

3. Preparation of the agar plates. The agar plates are prepared by pouring a sterile agar medium into sterile petri dishes and allowing it to cool and solidify. Once the agar medium has solidified, a bacterial culture is swabbed onto the agar surface, making sure that the entire surface of the agar plate is covered with bacteria.
4. Preparation of discs. Antimicrobial discs were prepared by punching out discs (6 mm in diameter) from filter paper using a sterile punch. These discs are then loaded with the prepared alcogels containing different hydrogels.
5. Inoculation of discs. The discs containing alcogels are then placed onto the agar surface that has already been inoculated with bacterial cultures. The plates are then incubated at the recommended temperature for 24 hours.
6. Measurement of zones of inhibition. After incubation, the plates are examined for zones of inhibition, which are areas of clear growth inhibition around the disc. The size of the zones of inhibition is measured in millimeters using a calibrated caliper.

In order to assess the effectiveness of the bioethanol-derived alcohol gel's antimicrobial properties, an interpretation guide is necessary.

Table 2. MIC or zone diameter value breakpoints and interpretive categories are established per CLSI (Clinical and Laboratory Standards Institute) document M23[5] for categories of susceptible intermediate, and resistant [(9)].

Interpretive Category	Breakpoints	
	MIC, μg/mL	Zone Diameter, mm
Susceptible	$\leq$ 4	$\geq$20
Intermediate	Aug-16	15-19
Resistant	$\geq$ 32	$\leq$14

7. Statistical analysis. The data collected from the experiment is analyzed statistically to determine the significance of the results. Analysis of variance (ANOVA) and t-test was used to analyze the data.

3 Results and Discussion

3.1 Viscosity of the Different Bioethanol-derived Alcohol Gels

Figure 1 shows the viscosity values of various treatments, with the Control treatment acting as the baseline for comparison. Viscosity refers to a fluid's resistance to flow, with higher values indicating a thicker, more resistant fluid. The Control treatment has a viscosity value of 7561.67 mPa·s, which sets a reference point for comparison with the other treatments.

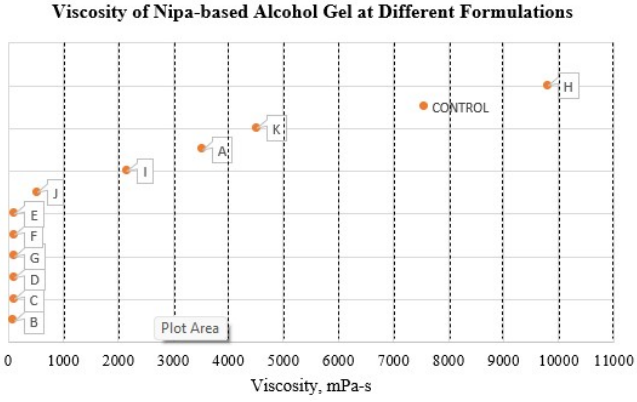


Fig 1. Viscosity of Nipa Alcohol Gel at Different Formulations (At room temperature)

The gelling agents used in the formulation of samples A, B, and C are Carbomer (Carbopol 940), carboxymethyl cellulose (CMC), and sodium alginate, respectively. On the other hand, Sample D, E, and F's gelling agents are a combination of carbomer and CMC, a combination of carbomer and sodium alginate, and a combination of CMC and sodium alginate, respectively. Figure 1 shows Sample B has the lowest viscosity value of 108.09 mPa·s followed by Sample C with a viscosity value of 108.43 mPas. The combinations of gelling agents specifically indicated in Treatments D, E and F did not significantly affect the increase

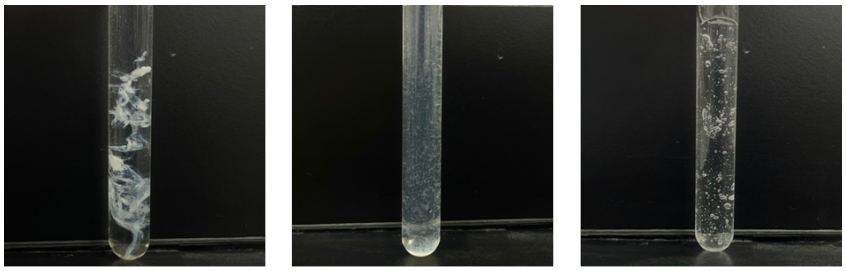
in viscosity. Comparing the 6 samples (A-F), sample A with 0.3g of the gelling agent carbomer showed the highest viscosity. The Carbomer or Carbopol 940 has more gelling properties and compatibility with the ethyl alcohol based on the viscosity values obtained. This phenomenon can also be observed in previous studies [10,11].

To further evaluate the compatibility of the gelling agents with the Nipa-based ethanol sample, the researchers conducted a qualitative evaluation of the different gelling agents at different concentrations of ethanol. From the quality control test in Table 3 and Figure 2, it was apparent that the gel formulations prepared carbomer indicated positive gelling even at low alcohol concentration (20%) and at the highest alcohol concentration (95%).

Table 3. Qualitative Evaluation of the Compatibility of the Different Gelling Agents with the Nipa-based Ethyl Alcohol at Varying Concentrations

Alcohol Concentration	Sodium Alginate	Carboxymethyl Cellulose	Carbomer
20%	+	+	+
40%	+	+	+
60%	-	-	+
80%	-	-	+
95%	-	-	+

+ Positive gelling with no presence of precipitate
— Negative gelling with the presence of precipitate



a. Sodium alginate b. Carboxymethyl Cellulose c. Carbomer

Fig 2. Visual Representation of the Different Alcogels Produced from different Gelling Agents

With the gelling agent resulting in the optimum viscosity, the percentage of the gelling agent carbomer (Carbopol 940), was used. The gel was prepared to vary the concentrations from 0.1%, where 0.4% of the carbomer. Sample G, A, and H contain 0.1g (0.1%), 0.3 g (0.2%w/w), and 0.5 g (0.4%w/w) carbomer, respectively. The viscosity was determined and compared to the commercially available hand sanitizer with a viscosity of 7561.67 mPa·s. Sample G, A, H resulted in a viscosity of 115.76 mPa·s, 3572.8 mPa·s, and 9808.8 mPa·s, respectively. Based on the viscosity, the control falls under the range of concentration from 0.3-0.5%w/w carbomer. In addition, it is observed that the higher the concentration of the carbomer, the higher the viscosity. This indicates that viscosity can be adjusted by the amount of gelling agent incorporated in the mixture.

Overall, the table suggests that Sodium Alginate and Carboxymethyl Cellulose are compatible with Nipa-based Ethyl Alcohol at lower concentrations (up to 40%), whereas Carbomer is compatible at higher concentrations (60% and above). At concentrations of 80% and 95%, none of the agents are compatible with the alcohol, as all result in the presence of a precipitate rather than a gel. However, it should be noted that only Carbomer did not result in the presence of a precipitate at alcohol concentrations between these concentrations. This finding is consistent with other studies [12–14].

3.2 Antimicrobial Assay using Disc-Diffusion Susceptibility Test

The experiment measured the effectiveness of each treatment by observing the size of the zone of inhibition around each treatment on a petri dish culture. The zone of inhibition is the area surrounding the treatment where bacterial growth is prevented, indicating the activity of the treatment against the bacterial strain. Samples containing less than 500 mPa·s of viscosity were excluded from the antimicrobial tests to prevent bias in the results.

Table 4 shows that the Control treatment exhibited some activity against both *S. aureus* and *E. coli*, with mean zone of inhibition values of 18.08 mm and 17.64 mm, respectively. However, Treatment A, D, H, I, and J all exhibited antibacterial activity against both strains, with mean zones of inhibition values ranging from 19.06 mm to 30.38 mm. Treatment A showed the highest activity against *E. coli*, with a mean zone of inhibition of 30.38 mm, indicating that this treatment was very effective against this strain. Treatment K exhibited activity against both strains as well, but only with mean zone of inhibition values of 14.22 mm for *S. aureus* and 16.85 mm for *E. coli*, which makes both strains resistant and intermediate, respectively.

Table 4. Antimicrobial Assay using Disc-Diffusion Susceptibility Test

Treatment	Test organisms	Mean Zone of Inhibition (mm)	Interpretation
CONTROL	*S. aureus*	18.08a	Intermediate
	E. coli	17.64c	Intermediate
A	*S. aureus*	23.35a	Susceptible
	E. coli	30.38a	Susceptible
H	*S. aureus*	21.64a	Susceptible
	E. coli	16.24c	Intermediate
I	*S. aureus*	19.06a	Intermediate
	E. coli	17.93c	Intermediate
J	*S. aureus*	15.13b	Intermediate
	E. coli	21.89c	Susceptible
K	*S. aureus*	14.22c	Resistant
	E. coli	16.85c	Intermediate
Level of Significance		**	

*Means with the same letter are not significantly different.

Based on these results, it can be deduced that treatments A, H, I, and J were very effective against both bacterial strains tested in the experiment, while Treatment K showed less activity but still had some activity against the bacteria. The high activity of these treatments may be attributed to the antimicrobial properties of the alcohols in the alcogel formulations, which are known to disrupt bacterial membranes and denature proteins.

Furthermore, treatment A showed the highest activity against *E. coli* with a mean zone of inhibition of 30.38 mm, indicating that it was very effective against this bacterial strain. Additionally, *S. aureus* was susceptible against Treatment A with a mean zone of inhibition value of 23.35 mm. Therefore, treatment A can be considered the most effective treatment among all the treatments tested in this experiment.

3.3 Relationship between the Formulation of Alcogels and their Antimicrobial Properties

Based on the data provided, we can see that Sample A, which contains carbomer, had the highest antibacterial efficacy against both *S. aureus* and *E. coli*, with a mean zone of inhibition values of 23.35 mm and 30.38 mm, respectively. Sample H, which also contains carbomer, had a relatively high antibacterial efficacy against *S. aureus* (21.64 mm) but a lower efficacy against *E. coli* (16.24 mm). Sample I, which contains carbomer and glycerol, had lower antibacterial efficacy than Sample A, but still showed activity against both *S. aureus* and *E. coli*. Sample J, which contains carbomer and fragrance, showed lower activity against *S. aureus* (15.13 mm) but higher activity against *E. coli* (21.89 mm) compared to the control. Sample K, which contains carbomer, fragrance, and glycerol, had the lowest antibacterial efficacy against both *S. aureus* and *E. coli* among all the samples.

When comparing the effect of fragrance and moisturizer on the antibacterial properties of the formulations, it can be seen that the addition of fragrance (Sample J) did not significantly enhance the antibacterial efficacy of the formulation. On the other hand, the addition of moisturizer (Sample I and K) did not show any clear trend in terms of the effect on antibacterial efficacy.

Overall, these findings suggest that carbomer may be a promising ingredient for formulating antimicrobial products, but the addition of fragrance and moisturizer may not necessarily enhance the antibacterial properties of the formulation.

3.4 Relationship between the Viscosity of Alcogels and their Antimicrobial Properties

The figures provide information on the viscosity and antibacterial activity of different alcogel treatments. The viscosity values indicate the thickness or consistency of each alcogel treatment, while the antibacterial activity shows the level of effectiveness of each treatment against bacterial growth.

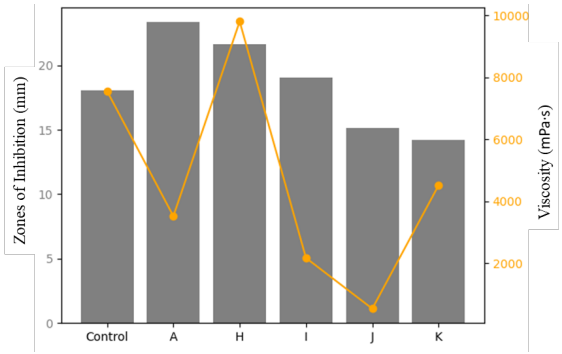


Fig 3. Correlation between Antibacterial Activity against *S. aureus*and the Viscosity of Alcogels

The Control treatment showed active antibacterial activity against the tested bacterial strains, indicating that it was effective in inhibiting bacterial growth. Treatment A, H, and I all showed very active antibacterial activity against the tested strains, indicating that they were highly effective in inhibiting bacterial growth. Treatments J and K showed active antibacterial activity against the tested strains, indicating that they were somewhat effective in inhibiting bacterial growth.

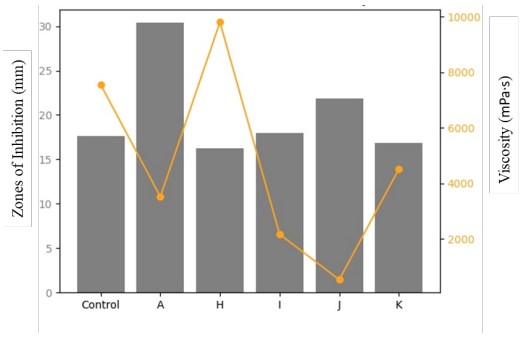


Fig 4. Correlation between Antibacterial Activity against *E. coli* and the Viscosity of Alcogels

When comparing the viscosity values of the treatments, it can be observed that Treatment A had a lower viscosity value compared to the other treatments. However, it also exhibited very active antibacterial activity against the tested strains, indicating that the lower viscosity did not negatively impact its antibacterial properties. Treatments J and K also had relatively low viscosity values compared to the other treatments but still exhibited active to very active antibacterial activity.

Treatment H had the highest viscosity value, indicating a thicker consistency compared to the other treatments. However, it still exhibited very active antibacterial activity against the tested strains, indicating that its thicker consistency did not hinder its antibacterial properties. The treatment I had a lower viscosity value compared to Treatment H but still exhibited active antibacterial activity against the tested strains.

Overall, the data suggest that the viscosity of alcogel treatments may not have a significant impact on their antibacterial activity. Some treatments with lower viscosity values exhibited very active antibacterial activity, while others with higher viscosity values also exhibited very active antibacterial activity. Studies carried out by Chojnacki, M., et al. (2021) and Sommatis, S., et al. (2023) provide examples of this phenomenon[15,16]. However, further studies may be needed to investigate the relationship between viscosity and antibacterial activity in alcogel treatments.

It is also important to note that the data only provide information on the effectiveness of these alcogel treatments against the tested bacterial strains. Further, studies may be needed to investigate their effectiveness against other bacterial strains and to evaluate their safety and suitability for use in different settings.

4 Conclusion

Based on the provided data and literature review, it can be concluded that the antimicrobial activity of alcogels is affected by various factors such as the type and concentration of the alcohol used, the presence of other antimicrobial agents, and

the viscosity of the gel. In the provided experiment, several treatments (A, H, I, J, and K) were found to exhibit significant antibacterial activity against two different bacterial strains, while Treatment B and C showed very low activity.

It is also worth noting that viscosity and its formulation seem to have a limited effect on the antibacterial activity of alcogels, as shown by the lack of correlation between viscosity and activity in the provided data. However, Treatment A exhibited the highest inhibition zones among all treatments. It has a formulation of 0.3 grams of carbomer mixed with 33.23 mL of distilled water, 2 drops of triethanolamine, and 116.67 mL of 90% ethyl alcohol. In addition, it has a viscosity of 3527.81 mPa·s. Furthermore, the data suggest that this treatment had the best formulation and viscosity against the two bacterial strains. However, more research is needed to assess their effectiveness against a wider range of bacteria and other pathogenic microorganisms.

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