Original Research Article

The Influence Of Viscosity On The Antimicrobial Activity Of Simple Alcoholic-Based Hand Sanitizer For Infection Of Covid-19 In Nigeria.

ABSTRACT

Aim: This study was carried out to analyze and evaluate the influence of viscosity on the antimicrobial activity of simple alcoholic-based hand sanitizer preparations.

Methods: The preparation of different viscosities of hand sanitizer was done by the introduction of carbomer to define its physicochemical stability. Two folds serial dilution of the test product using isopropyl alcohol and ethanol as alcohols and biocide efficiency to determine the antimicrobial activity using killing time assay were carried out.

Result: The pH was adjusted to 7.29 and 7.11 respectively, which shows the neutrality of the products. More so, the pH of the two test products proved to be good and hence, stable. In terms of viscosity, it decreased as the concentration decreased at 0.07% for most of the organisms which indicated good antimicrobial activity on the bacterial and fungi strain used. However, concentrations of isopropyl-formulated test product were more effective than the ethanol-based product on the bacterial strains, with Pseudomonas aeruginosa having the highest percentage of microbial death at 92.5%. Although, the ethanol-based products showed higher antimicrobial activity against fungi strains than the isopropyl test product.

Conclusion: Viscosity influences the activities of alcohol-based hand sanitizers since less viscous test product has a less concentration. Thus, more water denatures the proteins of the microorganism and limits the release of the active ingredient from the formulation. Sequel to this, viscosity enhancers like thickeners and gelling agents like carbomer should be used minimally in the formulation of these products.

***Keywords*:** Hand hygiene, formulations, biocide efficiency, microbial death

1. INTRODUCTION

Microorganisms are ubiquitous in nature and found to exist freely in nature. They exist in air, water, soil and other environments such as the skin, the digestive tract and orifices. They are quite beneficial as seen in microflora that naturally constitutes the human system for the proper functioning of the body. However, they could be a force to reckon with – an unseen enemy of man especially when their normal distribution in nature is disturbed. Microorganisms have led to a lot of health crises over the years. They cause infections and some other life-threatening diseases. As these health crises are constantly on the increase, there is an absolute need to keep these microorganisms in check.

Conventionally, antibiotics have been used to treat and control the havoc caused by microorganisms. However, this approach tends to be obsolete; this is because of the diminishing efficiency of antibiotics in treating infections as we are in post-era antibiotics (Gahrn-Hansen and Hornstrup, 1994). Hence, there is a need for a more subtle approach.

According to WHO (WHO 2009) and Pieres *et al.*, (2017), prevention of the spread and transmission of these pathogenic organisms by breaking the infection chain is an effective tool than trying to treat the diseases and infections that might occur. One of the techniques in achieving this includes good sanitation for example hand hygiene, and the use of antimicrobial agents such as antiseptics, sanitizers, disinfectants and so on. These techniques have been proven to control the number, inhibit the growth of the organisms or eventually kill the microbes (Pieres *et al.*,2017). For instance, hand hygiene is compliance with the cleansing of the hands with soap and water or with antiseptic hand rub to remove transient microorganisms from hands and maintain the condition of the skin (Engdaw *et al.*, 2019). Globally, hand hygiene has become an important healthcare issue and is the single most cost-effective and practical measure to reduce the incidence of nosocomial infections and the spread of antimicrobial resistance

(Abebe and Yeshanew, 2017). Aiello *et al.,* (2008) reported that hand hygiene caused a 31 % reduction in the incidence of gastrointestinal illness and about a 21 % reduction in respiratory illness globally.

In the same vein, the use of antiseptics and disinfectant has also been implicated in the prevention of diseases. They either inhibit or kill the growth of microorganisms without causing any adverse effect on the surfaces they are applied to. Hospitals and healthcare environments have benefited a lot from the use of antiseptics as they help to reduce the transient microbial flora on the hands of healthcare providers, reduce inter-person transmission of microbes, and to achieve surgical hand antisepsis (Weber *et al.,* 2007). Antiseptics like disinfectants kill the microorganisms completely and hence antiseptics are mostly classified based on their functional groups which could be alcohols, chlorine compounds, iodine compounds, quaternary ammonium compounds and so on (Jiang *et al.,* 2020). With this, their efficiency is determined by the class they belong to and some other factors such as pH, temperature, concentration, time of exposure and so on.

In non-pharmaceutical interventions such as in the case of Ebola and COVID-19, hand sanitizers have been shown to affect the virus and other pathogens (Pittet, 2001; Boyce and Pittet, 2002; Kampf and Kramer, 2004; Bloomfield, 2007 and Munayco *et al.,* 2009). As of now, hand sanitizers are in high demand mostly because of the scarcity of water in some regions which makes hand-washing facilities not readily available in public places (Beradi *et al.,* 2021). In this viewpoint, alcohol-based hand sanitizers (ABHS) are the most commonly used type of sanitizer. They contain about 60 to 95 % alcohol as recommended by WHO. Jiang *et al.,* (2020) stated that this form of hand sanitizer is the most effective and convenient to use against infection. Several studies have also reported that sanitizers with at least 70% alcohol were suggested to eliminate 99.9 % of the bacteria on hands (Rotter, 1999) after application within the first 15 s (Aiello and Larson, 2002). Alcohol-based hand sanitizers contain ethyl alcohol, isopropyl alcohol, propanol or a combination of the family of alcohols (Widmer 2000). They have a broad-spectrum tendency by disrupting the cytoplasmic integrity through protein coagulation, denaturation resulting in cell lysis and interference with cellular metabolism. Hence, they can be used to disrupt the infection cycle of microorganisms. This is particularly seen in the emergence of COVID-19 whereby alcohol-based hand sanitizers have been widely accepted and used by the general public.

However, many factors influence the efficiency of sanitizers. A study by Enwuru *et al.*, (2021) revealed that about 45% of the hand sanitizers had poor efficacy and were noted as quite high, considering the current state of the pandemic. A previous study by Russell (2004) also reported some of the factors that could lead to poor efficacy of the antimicrobial nature of hand sanitizers. Factors such as the quality and composition of the active ingredient, volume of hand hygiene and the inclination of the user to observe the proper procedure. He further argued that the bio-load, concentration of agent used, presence of biofilms, environmental pH, presence of organic matter and debris and much more are some of the factors that affect the antimicrobial nature of an alcohol-based hand sanitizer. To solve the problems of infections and resistance caused by these microbes, it is imperative to further analyze and evaluate the influences of some of the factors on the antimicrobial activity of alcohol-based hand sanitizers. This study aimed to evaluate the influence of viscosity on the antimicrobial activity of simple alcoholic-based hand sanitizer preparations. The findings could give more insights into how to maximize the efficiency of the sanitizers towards a wider range of organisms.

2. METHODOLOGY

2.1 Study design

This study was done between February and July 2021 and it is an experimental study design. Two gel formulations were made using different alcohol concentrations (85% ethanol and isopropyl alcohol) but containing the carbomer. Likewise, serial dilution was performed to vary the concentrations of each gel formulation. The antimicrobial activity in the control was maintained which also create a starting point for improving the formulation of each product to obtain efficient hand sanitizers. Furthermore, the culture media used include the nutrient agar, Sabouraud Dextrose Agar (SDA) and were all prepared according to the manufacturer's instructions.

2.2 Formulation

Two sanitizer products were prepared by mixing different ingredients in different percentages and mass

i. F1: This is the first product containing 85% ethanol, 100 ml of carbomer and triethanolamine

ii. F2: This is the second product containing 85% isopropyl alcohol, 100 ml of carbomer and likewise, triethanolamine to neutralize the mixture

iii. F3: Serial dilution was done to obtain a different concentration of each of the formulation

2.3 Evaluation of Hand Sanitizer stability

The organoleptic properties of the formulations were evaluated. The pH and viscosity were measured. pH values were brought close to neutrality using triethanolamine. For the evaluation of viscosity, a viscometer was used and readings were taken directly from the viscometer in mPa

2.3.1 In-vitro Biocide Efficiency

Biocide efficiency was evaluated using the kill time assay procedure. This test was done according to the standard guide for the assessment of antimicrobial activity using the time-kill kinetics procedure of the Antimicrobial Susceptibility Testing method with slight modifications (Reller *et al.*, 2009). Two different bacterial strains were exposed to the test substance – *Pseudomonas aeruginosa* and *Salmonella spp* and a fungi strain – *Tinea spp* was also exposed to the test substance. Plate count was used to determining the microbial population of the strains within a time frame.

Tubes containing 3 mL of each of the formulations and alcohol alone (positive controls) were inoculated with 0.1 mL of the standardized bacteria suspension called (a reaction mixture) to achieve a concentration of approximately 10^6 CFU/mL, this is used for the viable count, aliquots were removed from each of the reaction mixtures at specific time intervals(0,15,30,45,60 and 75 s) and plated onto the surface of corresponding sterile nutrient agar and sabouraud dextrose agar (SDA) and incubated at 37 ^{o}C for 18 to 24 hr for bacteria and 25-28 ^{o}C for 72 - 96 hr.

After the incubation time, the number of viable organisms was counted in CFU/plates. The test substance was considered biocide when there is a reduction in the colony-forming units

2.4 Data Analysis

Data were analyzed using the linear regression model to determine the level of correlation between the parameters.

3. RESULTS AND DISCUSSION

The chemical and physical properties of each of the formulations showed all most the same organoleptic characteristics based on smell and appearance. These properties prove that the formulations were stable, see, Table 1. The pH values were measured using the pH meter. Triplicates measurement was taken and the mean was calculated for the triplicate measurements. From Table 1, it can be seen that the initial pH for both products was slightly acidic. This value seems too acidic even for the skin according to Ningsih *et al.*, (2017). However, triethanolamine was used to adjust the pH to bring the pH close to neutrality. These values are in line with the work of Hasyim and Baharudin (2011) who reported that pH values greater than 6.5 can be tolerated by the body.

The viscosity of the formulated products showed a varying viscosity after making serial dilutions to obtain concentrations of 1.1%, 0.55%, 0.28%, 0.14% and 0.07%. The results for the viscosity were calculated as the mean of the three-sample measurement and represented graphically in Figure 1 and Figure 2. For the isopropyl formulated product (F2), the highest viscosity value was at a concentration of 1.1% (30.6 mPa) while the lowest viscosity was at a concentration of 0.07 % (0.1 mPa), see Figure 1. This shows that viscosity decreases with a decrease in concentration. This trend was also observed in the ethanol-formulated hand sanitizer, see Figure 2.

For the in-vitro biocide efficiency using the kill assay procedure to check for the reduction of viable cells of the bacterial strains and fungi strains. Previous studies (Fallica *et al.*, 2021) have reported that a substance is considered biocide when there is a reduction in the viable cells of at least 10^5 after a maximum of 5 min for a bacterial strain and 15 min for a fungi strain. However, in this study, it was observed that the highest microbial death for *Salmonella typhi*, was 88% using F2, which occurred at 75secs, and 70.3% at time 75secs. The highest percentage of microbial death occurred at a concentration of 0.07% with no reduction observed with the positive control using ethanol and a microbial death of 51.7% at 75secs using 85% IPA as a positive control, see Figures 3 and 4.

For *Pseudomonas aeruginosa*, it was observed that the highest microbial death for *Pseudomonas aeruginosa* was 98% for F2, which occurred at 75sec and 92.7% for F1 at time 75secs with both positive controls of the alcohols, for the diluted test products, the concentration of 0.07% had the highest percentage of microbial death occurring at 75 s with 84.2% and 77% for F2 and F1 respectively, see Figure 5 and 6. Likewise, for *Tinea spp.*, it was observed that the isopropyl-formulated product gave a reduction of 73% with a positive control of 85% at 15 s contact, see figure 7. 71% viability reduction was also seen in the ethanol-formulated product at 75 s contact, see figure 8. 0.0625% has the highest percentage of microbial death occurring at 75 s with 84.2 % and 77 % for F2 and F1 respectively.

Alcohol-based hand sanitizer which could be in form of liquid, gel or foam is commonly used to inactivate microorganisms and/or temporarily suppress their growth when applied on the hands. It is a form of a good hand hygiene routine that is considered useful in both hospital and community settings (Kratzel *et al.,* 2020). Due to the high scarcity of water in some regions, alcohol-based hand rubs (ABHRs) are highly in demand and have been proven to be the most effective, convenient infection preventive measure (Hadaway, 2020). However, their efficacy is mostly affected by some factors such as the presence of some additives especially those that can cause an increase in their viscosity (Nzekwe *et al.,* 2018).

In this study, the influence of viscosity on the antimicrobial activity of simple alcohol-based hand sanitizer. Data analysis has shown a decrease in the plot of viscosity against concentration. It was observed that viscosity decreased with the concentration, with the least concentration having better antimicrobial activities. Previous studies by Suchomel *et al.,* (2013) and Nzekwe *et al.,* (2018) have all shown the effect of high concentrations of additives that could increase the viscosity of sanitizers while reducing their activity. Nzekwe and Colleagues in 2018, observed that the increase in viscosity of the samples due to the high concentrations of glycerin caused an inhibition of the diffusion of the product through the culture medium.

However, studies by Ochowoto *et al.*, (2015) revealed that less viscous products had better activity compared to gel-based products which was also in line with the study conducted by Enwuru *et al.*, (2021). In this study, the antimicrobial activity of the product formulated with IPA on *Salmonella typhi* highest reduction in viable cells at a concentration of 0.07 % with a microbial death of 88% at 75 s contact time. This could be a result of less viscosity of this concentration which increases its tendency to release its active ingredient leading to increased biocidal activity. Furthermore, the ethanol-formulated product, *salmonella type*, had the highest percentage of microbial death observed at 41.7%, 80%, 59.3%, 0 % and 70.3% with no microbial death observed for the positive control 85% ethanol without carbomer. When the concentrations of the carbomer were compared, 0.5% had the highest microbial death of 80% at 75 s. In comparison with the positive control, the test products had a good activity than the positive control.

For the ethanol-based product against *Pseudomonas aeruginosa*, 97.7 % microbial death was observed with the control but an effective concentration of 0.07 % gave the best activity of 84.2% at 75 s. Also, 98 % microbial death was observed with the positive control for an isopropyl-formulated test product against *Pseudomonas aeruginosa* with an effective concentration of 0.07 % resulting in 92.5 % at 15 s contact time. This observation is in line with the work done by Nzekwe *et al.*, (2018) with isopropyl-formulated products having an edge over ethanol in terms of the spectrum of activity.

The fungi strain, *Tinea spp*, was also tested against the isopropyl-formulated product and ethanol-formulated product. For the positive control, 73 % of the *Tinea spp* were killed, however, when the concentrations of the carbomer were compared, 0.07 % had the highest reduction of viable cells (microbial death) of 70% at 75 s contact time. The ethanol-formulated products gave a less 60 % reduction in the positive control with the carbomer concentration of 0.28% having the best activity of 75.6 % reduction at 30 s contact time.

Consequently, when taking all these into account, it is reasonable to say that a test product formulated with isopropyl alcohol has a better bactericidal activity as seen in *Pseudomonas aeruginosa* than an ethanol-based test product. However, ethanol-formulated products have better activity on the fungi strains (*Tinea spp)* which is in line with previous studies.

This study had some limitations. It does not precisely follow the WHO Formulation 1 but was done only to determine, formulate and evaluate two test products that could have a higher antimicrobial activity when viscosity is concerned.

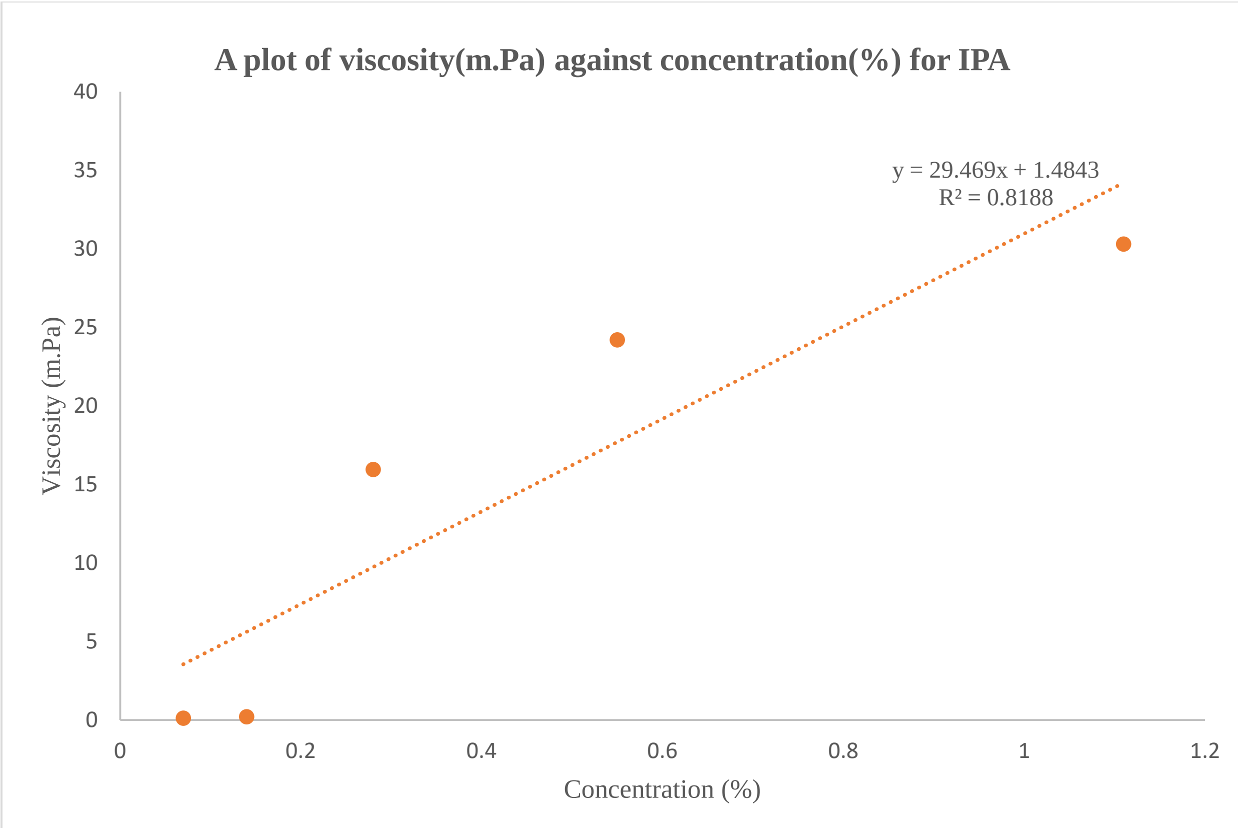

Figure 1: A linear graph showing the viscosities of the various concentrations of the formulated with isopropyl alcohol

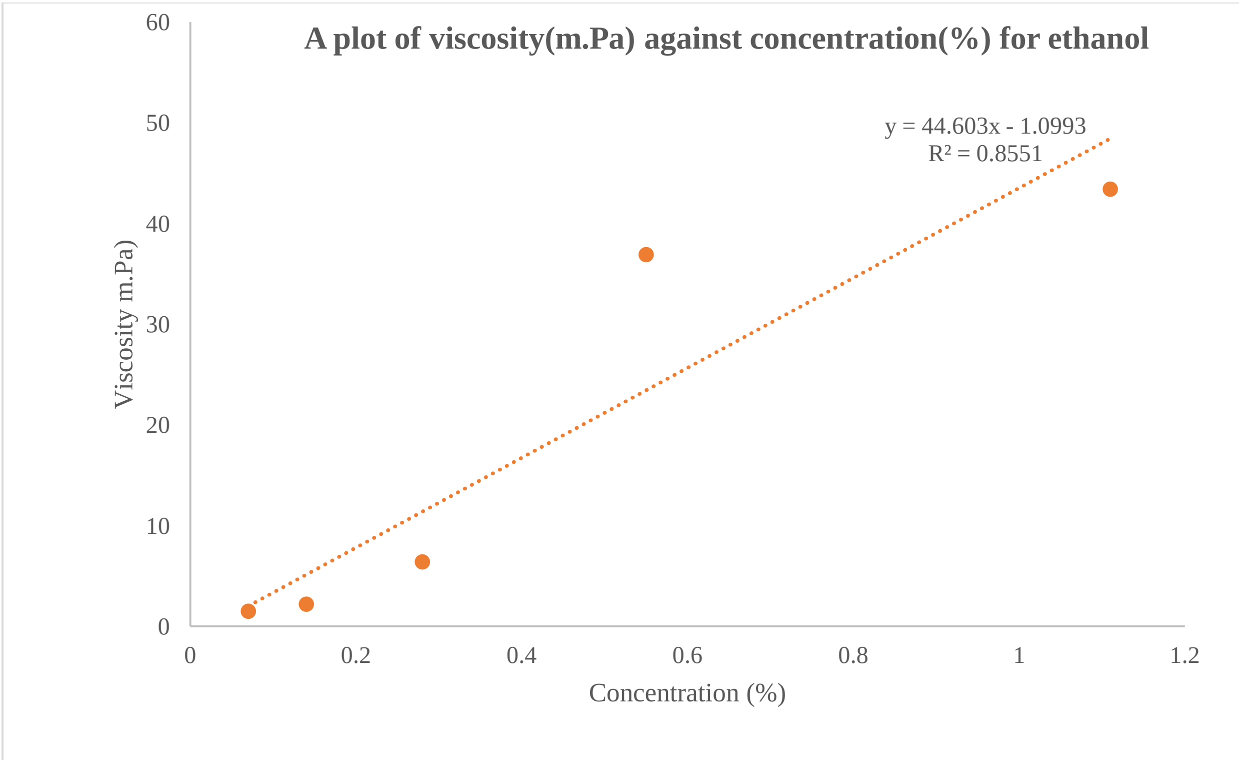

Figure 2: A linear graph showing the viscosities of the various concentrations of the formulated product ethanol

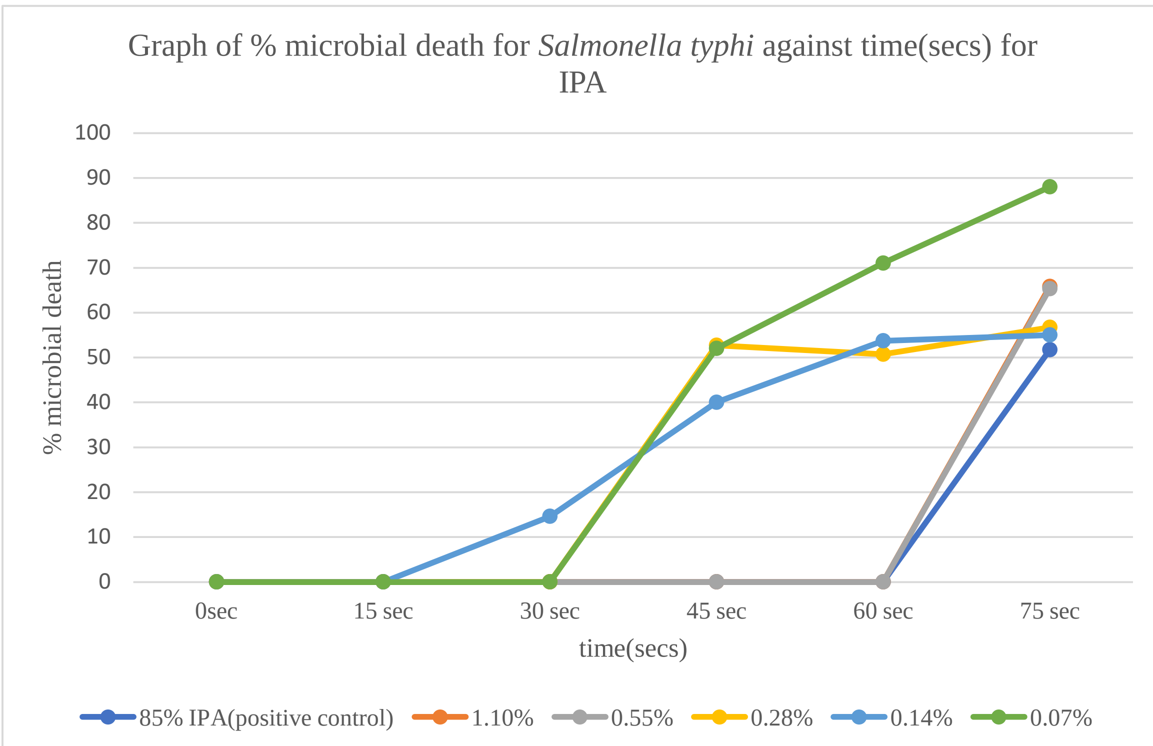

Figure 3: A graph showing the percentage of microbial death for *Salmonella typhi* against time for isopropyl alcohol.

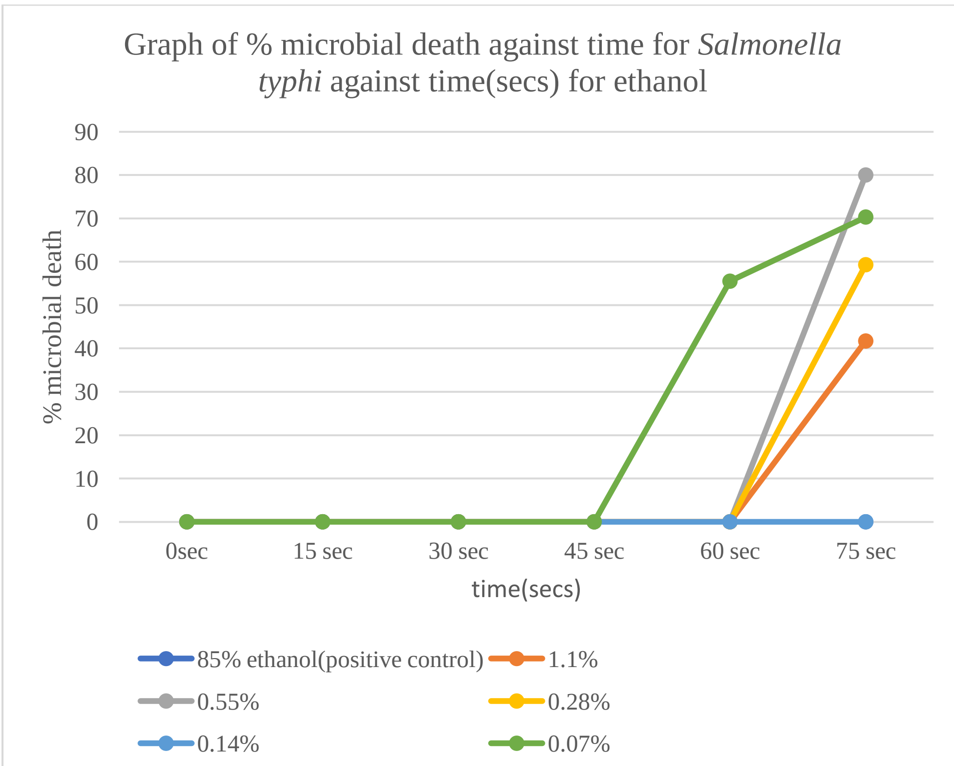

Figure 4: A graph of the percentage of microbial death against time for Salmonella typhi against time for ethanol

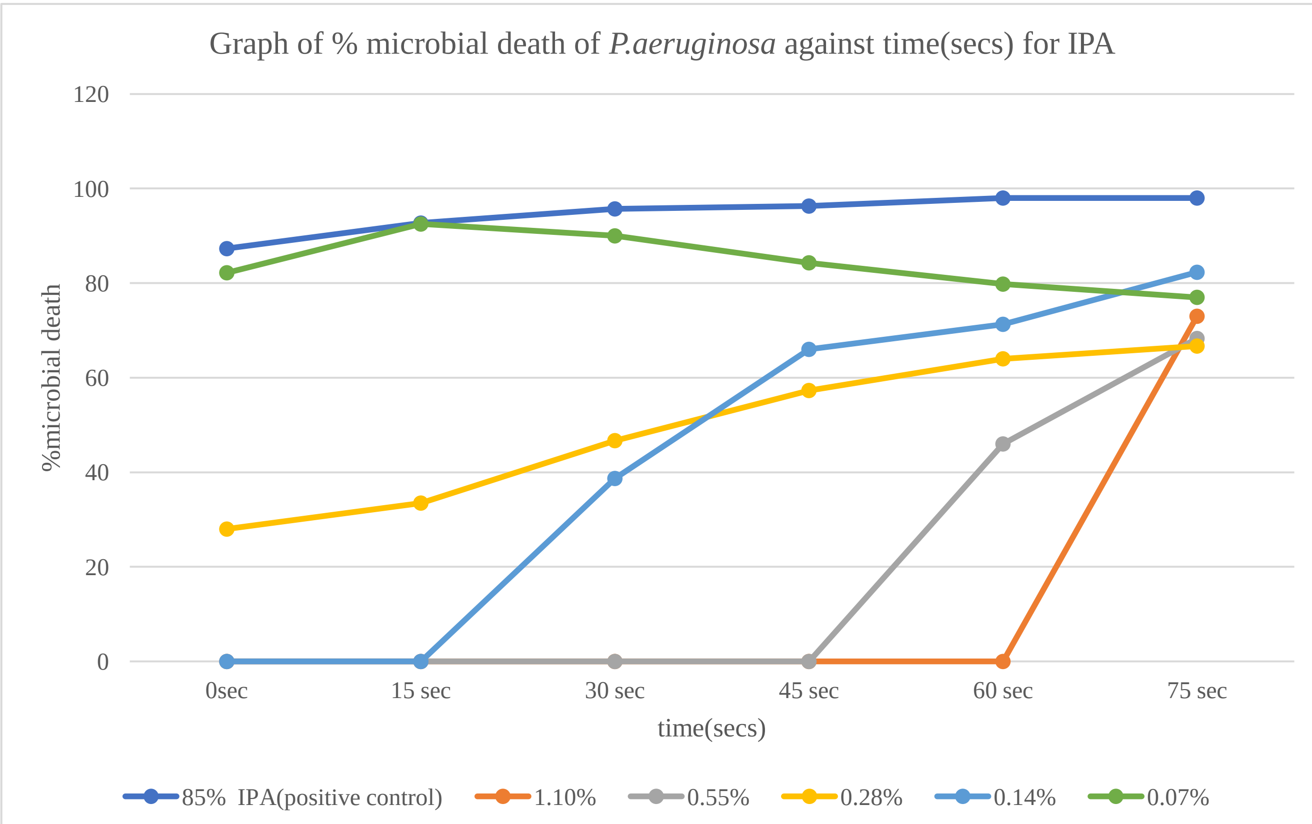

Figure 5: A plot of the microbial death of *Pseudomonas aeruginosa* with isopropyl alcohol.

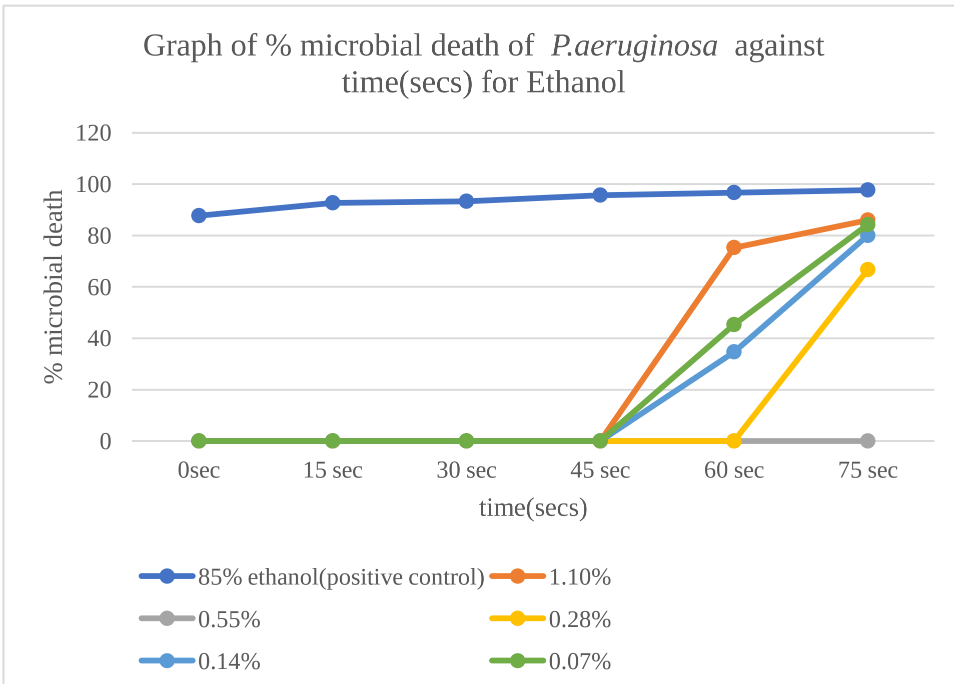

Figure 6: A plot of the percentage of microbial death for *Pseudomonas aeruginosa* for ethanol.

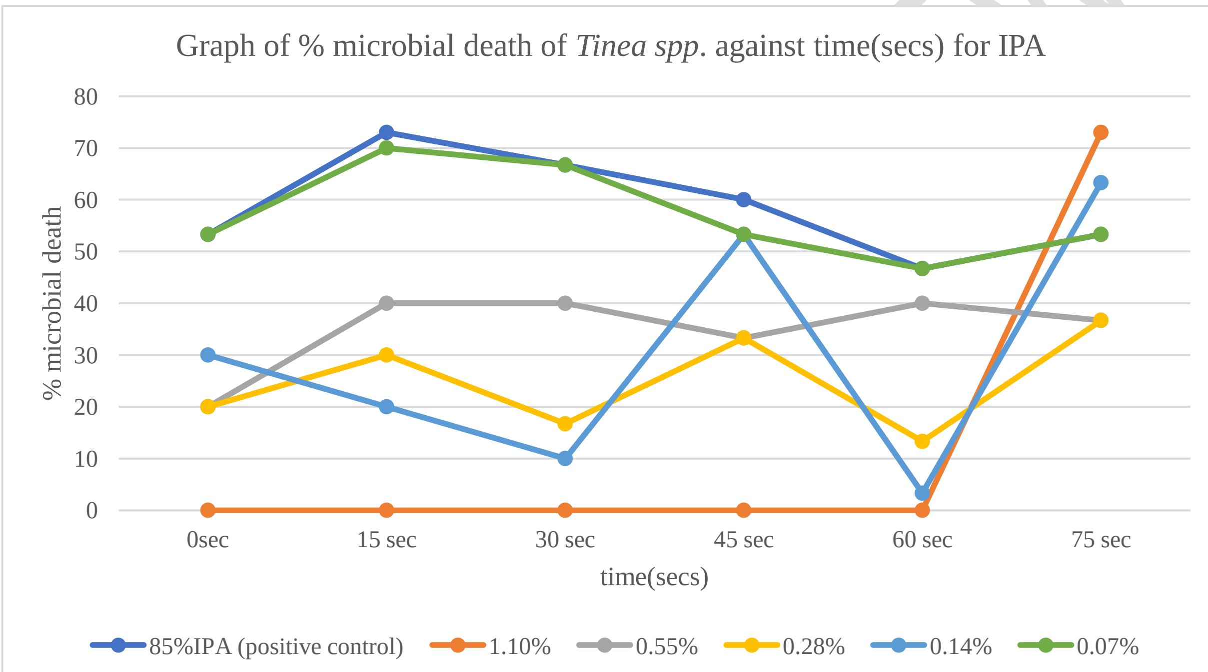

Figure 7: A plot of the percentage of microbial death of *Tinea spp* with IPA

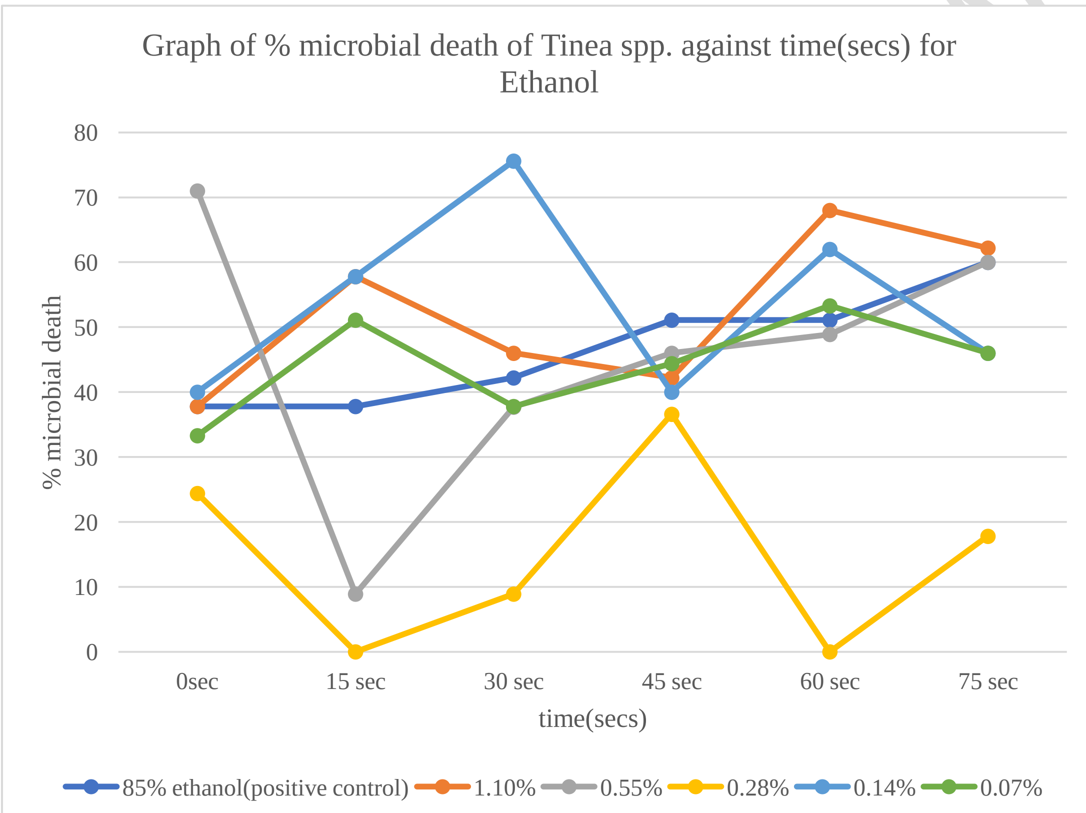

Figure 8: A plot showing the percentage of microbial death for *Tinea spp* with ethanol

Table 1: The organoleptic properties, pH, and carbomer percentage of the two products

Products	Colour	Odour	Initial pH	Final pH	% Carbomer
F1	Transparent	Properties of alcohol	2.90	7.29	2.5% ethanol carbomer
F2	Transparent	Properties of alcohol	2.85	7.11	2.5% isopropyl carbomer

4. CONCLUSION

The concentration of alcohols in the formulation of ABHS is important for its activity and should always be within the range of 60 to 95% as recommended by WHO. The hand sanitizer formulated with isopropyl alcohol was considered to have better activity compared to ethanol. The viscosity decreased with the concentration, with the least concentration having better antimicrobial activities because at this point the product was less viscous permitting the release of more of the active ingredients causing protein denaturation with the presence of water. Thus, we conclude that viscosity could influence the antimicrobial activity of ABHS and as such should be carefully considered during formulation to avoid altering the activity of the final product.

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